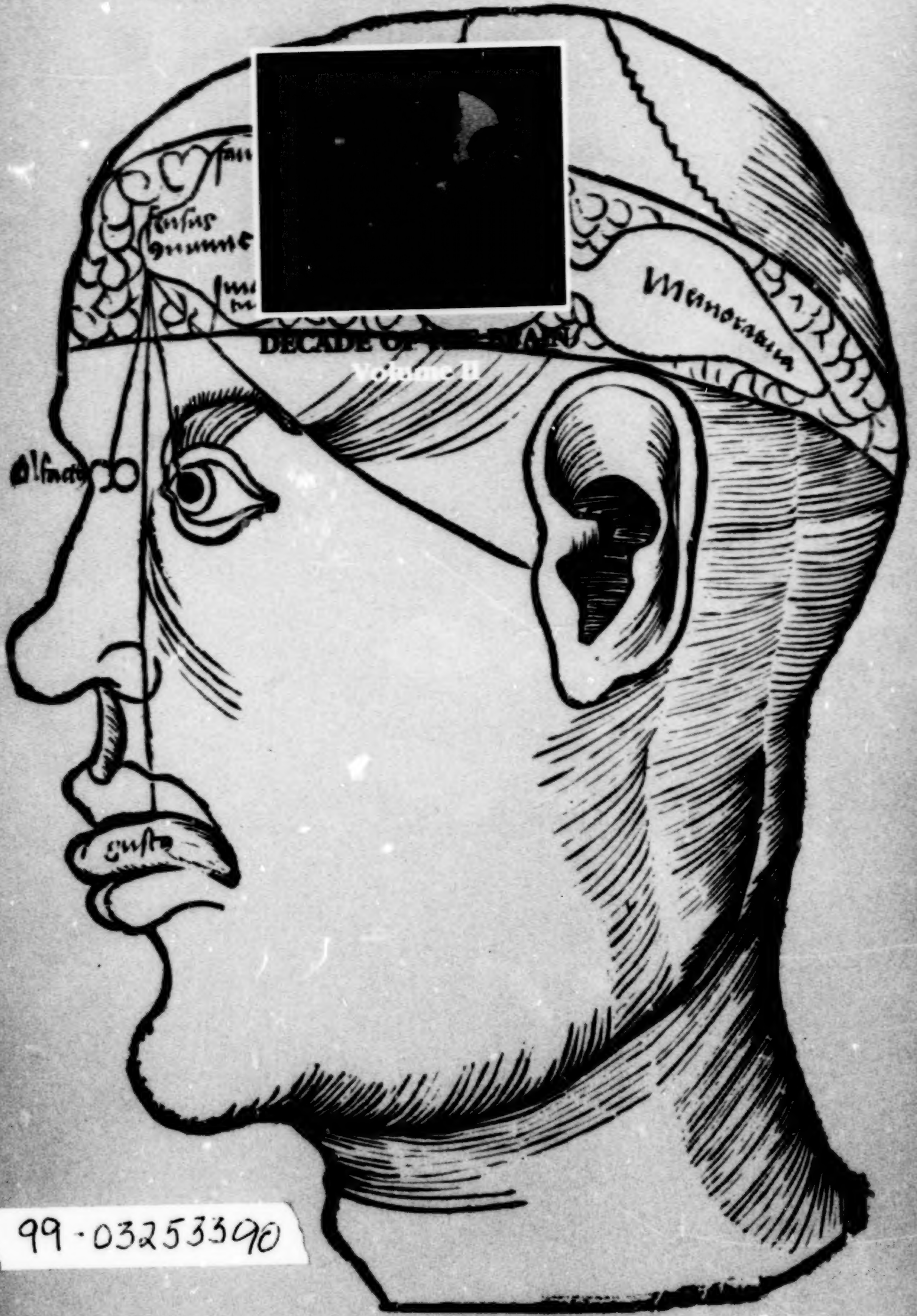
THE ADAPTABLE BRAIN



DECADE OF THE BRAIN

Volume II

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THE ADAPTABLE BRAIN

Papers presented at a symposium series
cosponsored by the
National Institute of Mental Health
of the
National Institutes of Health
and the
Library of Congress

Sherry Levy-Reiner
COORDINATOR
PROJECT ON THE DECADE OF THE BRAIN
Volume Editor

DECADE OF THE BRAIN
Volume II

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Washington 1999

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Library of Congress Cataloging-in-Publication Data

The adaptable brain : papers presented at symposia cosponsored by
the National Institute of Mental Health and the Library of Congress
/ Sherry Levy-Reiner, coordinator.
p. cm. — (Decade of the brain ; v. 2)
Includes bibliographical references and index.
ISBN 0-8444-00971-5 (pbk. : alk. paper)
——— Copy 3. Z663 .A832 1998
1. Neuroplasticity—Congresses. 2. Brain—Adaptation—Congresses.
3. Neurosciences—Congresses. I. Levy-Reiner, Sherry.
II. National Institute of Mental Health (U.S.) III. Library of
Congress. IV. Series.
QP363.3.A33 1998
612.8'2—dc21 98-41659
CIP

Cover: The logo for the LC/NIMH Project on the Decade of the Brain,
a modern representation of parts of the brain as revealed by
brain-imaging techniques, is superimposed on "Animae Sensitivae,"
an illustration from Gregor Reisch, ***Margarita Philosophica*** (1503),
Rosenwald Collection 595, Rare Book and Special Collections
Division, Library of Congress.

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402

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Volume II

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Funding for this lecture series project was provided by the National Institute of Mental Health, National Institutes of Health, with program support by the Library of Congress under interagency agreement no. Y01–MH–10050.

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PROCLAMATION 6158
DECADE OF THE BRAIN
1990-99

July 17, 1990

By the President of the United States of America

A PROCLAMATION

The human brain, a three-pound mass of interwoven nerve cells that controls our activity, is one of the most magnificent—and mysterious—wonders of creation. The seat of human intelligence, interpreter of senses, and controller of movement, this incredible organ continues to intrigue scientists and laymen alike.

Over the years, our understanding of the brain—how it works, what goes wrong when it is injured or diseased—has increased dramatically. However, we still have much more to learn. The need for continued study of the brain is compelling: millions of Americans are affected each year by disorders of the brain ranging from neurogenetic diseases to degenerative disorders such as Alzheimer's, as well as stroke, schizophrenia, autism, and impairments of speech, language, and hearing.

Today, these individuals and their families are justifiably hopeful, for a new era of discovery is dawning in brain research. Powerful microscopes, major strides in the study of genetics, and advances in brain imaging devices are giving physicians and scientists ever greater insight into the brain. Neuroscientists are mapping the brain's biochemical circuitry, which may help produce more effective drugs for alleviating the suffering of those who have Alzheimer's or Parkinson's disease. By studying how the brain's cells and chemicals develop, interact, and communicate with the rest of the body, investigators are also developing improved treatments for people incapacitated by spinal cord injuries, depressive disorders, and epileptic seizures. Breakthroughs in molecular genetics show great promise of yielding methods to treat and prevent

Huntington's disease, the muscular dystrophies, and the other life-threatening disorders.

Research may also prove valuable in our war on drugs, as studies provide greater insight into how people become addicted to drugs and how drugs affect the brain. These studies may also help produce effective treatments for chemical dependency and help us to understand and prevent the harm done to the preborn children of pregnant women who abuse drugs and alcohol. Because there is a connection between the body's nervous and immune systems, studies of the brain may also help enhance our understanding of Acquired Immune Deficiency Syndrome.

Many studies regarding the human brain have been planned and conducted by scientists at the National Institutes of Health, the National Institute of Mental Health, and other Federal research agencies. Augmenting Federal efforts are programs supported by private foundations and industry. The cooperation between these agencies and the multidisciplinary efforts of thousands of scientists and health care professionals provide powerful evidence of our nation's determination to conquer brain disease.

To enhance public awareness of the benefits to be derived from brain research, the Congress, by House Joint Resolution 174, has designated the decade beginning January 1, 1990, as the "Decade of the Brain" and has authorized and requested the President to issue a proclamation in observance of this occasion.

Now, Therefore, I, George Bush, President of the United States of America, do hereby proclaim the decade beginning January 1, 1990, as the Decade of the Brain. I call upon all public officials and the people of the United States to observe that decade with appropriate programs, ceremonies, and activities.

In Witness Whereof, I have hereunto set my hand this seventeenth day of July, in the year of our Lord nineteen hundred and ninety, and of the Independence of the United States of America the two hundred and fifteenth.

George Bush

[Filed with the Office of the Federal Register, 12:11 p.m., July 18, 1990]

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Foreword

This is the second volume of papers from a continuing series of symposia sponsored by the National Institute of Mental Health (NIMH) of the National Institutes of Health and the Library of Congress. Like ***Neuroscience, Memory, and Language***, this second volume, ***The Adaptable Brain***, reflects a creative collaboration between two distinguished federal agencies. Its publication comes at a momentous time in the lives of both institutions.

The National Institute of Mental Health is marking its fiftieth anniversary during a period of wide-ranging public discussion of mental health issues. Our increasing understanding of how the brain functions is producing fundamental changes in how we think about mental illness. We now recognize that all mental processes are biological and that all illnesses that affect thought and behavior ultimately reflect some aspect of brain biology gone awry. This radical rethinking affects us personally as individuals in relationship to one another. It also affects us collectively as a nation evolving policies that will ensure all citizens' welfare and progress. This neural basis of thought, emotion, and behavior, however, is not taken to connote biological determinism.

One strand that binds the research in this volume is the brain's ability to adapt. The brain is ***plastic***, a technical concept. More impressively, it is a living and continuously adaptive organism, and because each of our brains responds uniquely to millions of experiences and stimuli, no two individuals in the world are alike. The result is a remarkable potential for human creativity, invention, and aspiration.

The Library of Congress is, also at the present time, using new technology to improve and expand its service to Congress and the nation. Current initiatives that build upon rapid advances in computer storage and software permit the Library to lead the way in disseminating comprehensive information and high-quality content.

The Library's ability to inform and to educate is greatly enhanced as it uses the global electronics network to provide extraordinary access to its collections and programs. Further information about elements of the papers in this volume, for example, is now available anywhere in the world at the touch of a few keys.

The Decade of the Brain is more than half over. More people have learned a great deal more about the brain since President George Bush signed a proclamation recommended by a Joint Congressional Resolution to designate the 1990s as the "Decade of the Brain." Yet we are still only at a very early stage in our exploration of what there is still to know about this most complex structure.

The lecture series that the Library of Congress and NIMH initiated in 1991 has highlighted some of the most exciting and significant research in molecular biology and in neuropsychology, in developmental neurobiology, in behavioral genetics and molecular genetics, in biophysics and in chemistry, in anatomy, in cognitive psychology and cognitive neuroscience, in neuroimaging, in artificial intelligence, and in linguistics: all fields in which the brain is being studied. The brain's complexity demands this kind of cross-disciplinary approach.

This second volume recapitulates lectures that occurred between 1993 and 1995. The subjects they address reflect ways in which our new knowledge is being applied to the treatment of some of the most devastating brain disorders. The lectures and papers published here thus reflect the perspectives and expertise of both the National Institute of Mental Health and the Library of Congress. Though NIMH directs its resources primarily to the conduct and support of brain and behavioral research, it also plays a leadership role within the federal biomedical research establishment in developing educational programs aimed at keeping the public abreast of revolutionary advances in brain and behavioral sciences.

Although the Library's primary mission is to respond to requests by the Congress and others for answers to immediate questions and guidance on specific issues, it also has the capacity and resources to examine whole fields that interact with society and have an impact on the communities in which we live and work.

We are delighted that this volume—along with other published materials, videotapes, sites on the World Wide Web (NIMH's home page: http://www.nimh.nih.gov/; the Library's home page: http://www.loc.gov/), and public programs—advances that potential by disseminating this important information to a wide and intensely interested audience.

James H. Billington The Librarian of Congress	Steven E. Hyman, M.D. Director National Institute of Mental Health National Institutes of Health

The Adaptable Brain

Antonio R. Damasio is Van Allen Distinguished Professor and head of the Department of Neurology at the University of Iowa College of Medicine. Born in Portugal, he received both his M.D. and his Ph.D. from the University of Lisbon. He joined the University of Iowa faculty in 1975 and also was appointed an adjunct professor at the Salk Institute for Biological Studies. In 1990, he received the William Beaumont Prize from the American Medical Association in recognition of his contributions to medical science through neuropsychological research.

Over the past decade, Dr. Damasio's research team has contributed to the understanding of vision, memory, language, and decision-making. The laboratories that he and his wife, Hanna Damasio, created at the University of Iowa are a center for investigating the neural substrates of mental functions based on neurological lesions. Dr. Hanna Damasio, a neuroscientist, specializes in brain-scanning techniques. Working with the victims of strokes and accidents, the two doctors have developed a number of pioneering theories about how the brain processes language and information.

In his book *Descartes' Error* (New York: G.P. Putnam, 1994), Antonio Damasio explores ideas about how emotions are processed in the brain. His 1995 presentation at the Library of Congress described his experimental neuropsychological studies in humans. Dr. Damasio has found that emotion and feeling, the learning and recall of factual knowledge, and social behavior are interrelated processes that depend on separate brain systems continuously adapting to new stimuli throughout the human life span.

EMOTION AND DECISION IN THE ADAPTABLE BRAIN

Antonio R. Damasio and Hanna Damasio

The understanding of any living tissue requires investigations at multiple levels of structure, from molecules and individual cells to ensembles of cells. The nervous system and its central division, the brain, is no exception and is actually more complex than any other living tissues. Not only are there molecules and cells (neurons) to contend with, but the parts of the neurons—axons, dendrites, cell bodies—are also important. Moreover, the presumed 10 billion neurons of the human brain form circuits at multiple spatial scales, all the way from local and microscopic to nonlocal and macroscopic. Some of the latter actually span the entire brain and are called *large-scale systems.*

Understanding the brain's function is indispensable to the understanding of mind (cognition) and behavior, and understanding the brain's function also is essential to managing effectively neurological and psychiatric diseases. Such understanding requires multiple scientific efforts applied to all levels of brain organization. In other words, we cannot fully understand learning and memory without knowing what goes on—in terms of the interaction of molecules—at the level of axons, dendrites, and the synapses they form.

Some of these interactions are controlled by genes. Knowing that gene expression is required for, say, consolidating a particular memory will not, however, tell us which large-scale systems are necessary to construct or retrieve that memory in the brain. In short, if we are to make sense of what the 10 trillion synapses our neurons form are up to, we need to know it all.

Until quite recently, the most successful efforts to understand brain function focused on cellular and subcellular levels of organization. The techniques available to approach those levels were more advanced than the ones available for the large-scale systems more closely linked to the mind phenomena that make us

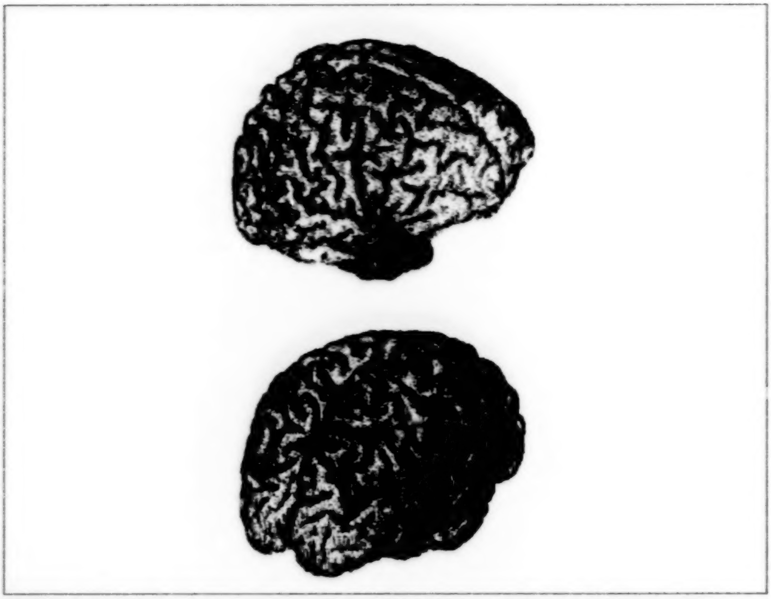

Figure 1. Example of the reconstruction of the human brain of a living normal person. The reconstruction used the technique known as BRAINVOX developed by Hanna Damasio and Randall Frank and is based on the computer manipulation of raw data from high resolution magnetic resonance scans. From the Human Neuroanatomy & Neuroimaging Laboratory, Department of Neurology, University of Iowa College of Medicine.

human. That situation has changed with the advent of powerful new techniques that allow us to approach those higher order systems not just in animals but also in humans. Some of these techniques, such as magnetic resonance imaging (MRI), have helped redefine the lesion method, whereas positron emission tomography (PET) and functional MRI (fMRI) permit us to catch the normal human brain on the fly, as it were, and get a more dynamic glimpse of its workings. Together with developments in cognitive science and theoretical neuroscience, these new techniques enable us to carry out a basic science effort in humans. The effort completes and extends traditional clinical research in both neurology and psychiatry (figure 1).

Adaptive human behavior requires innate knowledge that depends on both genetic instructions and real-life interaction during development; it also requires knowledge about novel situations that is acquired as life goes on, long after the basics of development are complete. The result is a constant change in brain circuitry that enables us to record new aspects of the ongoing interactions between organism and environment. The result is also a constant remodeling, at least in part, of the circuitries as they existed before a new interaction.

To illustrate what we mean, we will describe a task developed by one of our collaborators, Antoine Bechara. A player, who can be either a normal individual or a neurological patient, sits in front of four decks of cards similar in appearance and size and is given a $2,000 loan of facsimile U.S. bills. The player is told that the task requires a series of card selections, one card at a time, from any of the four decks.

After turning each card, the player receives some money, the amount of which is announced only after the turning and varies with the deck. After turning some cards, the player is given money and asked to pay a penalty; again, the amount is announced only after the card is turned and varies both with the deck and the card's position in the deck according to a schedule unknown to the player.

The player is told that the goal of the task is to maximize profit on the loan of play money and he or she is free to switch from any deck to another at any time and as often as wished, but the player is not told in advance how many card selections must be made. (In fact, the player is stopped after making 100 card selections.)

Turning any card from deck A or deck B pays $100; turning any card from deck C or deck D pays $50. The ultimate future yield of each deck varies, however, because the penalty amounts are higher in the high-paying decks (A and B), and lower in the low-paying decks (C and D). For example, after turning ten cards from deck A, the player has earned $1,000 but also has encountered five unpredicted punishments costing $1,250, thus incurring a net loss of $250. The same happens if the player selects from deck B. In contrast, turning ten cards from decks C or D earns the player $500, but the total unpredicted punishment is only $250, thus yielding a net of $250.

In short, decks A and B are equivalent in terms of overall net loss over the trials. The difference is that in deck A the punishment is more frequent but of smaller magnitude, whereas in deck B the punishment is less frequent but of higher magnitude. Decks C and D are equivalent in terms of overall net loss. In deck C, the punishment is more frequent and of smaller magnitude, whereas in deck D the punishment is less frequent but of higher magnitude. Decks A and B are thus "disadvantageous" because they cost the most in the long run, and decks C and D are "advantageous" because they result in an overall gain in the long run.

This task is a lot like life: there is complete uncertainty as to the outcome, and the rules of the game are acquired gradually by experi-

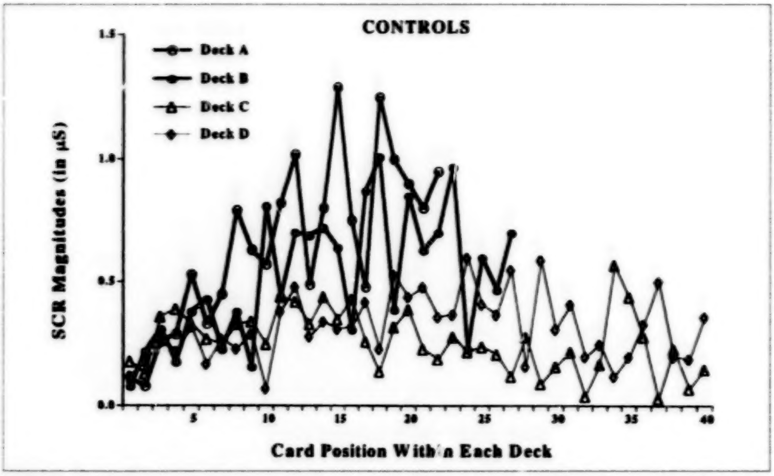

Figure 2. Graph showing the results of averaging of skin conductance responses, in twelve normal subjects, during the gambling task described in the text. There is a gradual increase in the skin conductance responses (SCR) to the bad decks (decks A and B) during the anticipatory period. The good decks do not lead to a buildup of skin conductance responses.

ence. One's acquisition of such knowledge is, however, influenced by the intrinsic values of the system: for example, one's preference of gain over loss, one's sense of the worth of money, and so on.

How do normal people behave at this task? They begin by sampling from the different decks, searching for clues as to how they should decide. At some point within the first twenty card choices, normal players begin to prefer decks C and D. At that point, they still have no concept of the structure of the task and thus are not making a conscious decision regarding goodness and badness. Yet they persevere in their advantageous choice. At some point beyond halftime, their choices become deliberate and conscious, and the players are fully cognizant of the secrets of the tasks—C and D, but not A or B.

Now consider what happens when—in addition to the behavior measures concerning deck choice, gains, and losses—we also measure a physiological indicator of the player's behaviors; we continuously can monitor, for example, skin conductance resistance while the subject is playing.

Figure 2 shows the result of averaging skin conductance responses (SCR) for twelve normal subjects during the period just before their actual selection of a card, that is, just as they are reasoning and making their decisions.

The results demonstrate clearly that long before the player is cognizant of the rules of the game, the player's brain is generating a powerful signal that closely correlates to the value of the decks. That is, the brain—but not yet the mind—is learning about the relationship between certain objects in the world (decks A and B) and the negative value of those objects. This is a palpable example of learning, of plasticity, of adaptation to circumstances, of the gradual acquisition over time of information that can be used nonconsciously (or eventually consciously) to steer behavior that is conducive to survival.

What systems in the human brain support this kind of learning? Is this the kind of learning that depends on the hippocampus, as does the learning of a new face or a new name, or a different place in the brain?

In fact, the nonconscious acquisition of this linkage depends critically on some cerebral cortices in the frontal lobe and a set of subcortical nuclei in the depth of the temporal lobe known as the amygdala. How do we know this and what does it mean?

Patients with damage to the ventral and medial part of the frontal lobe develop a most intriguing condition. Despite previously normal personal and social behavior, and despite preserved intelligence, language, and motor abilities, they become unable to make

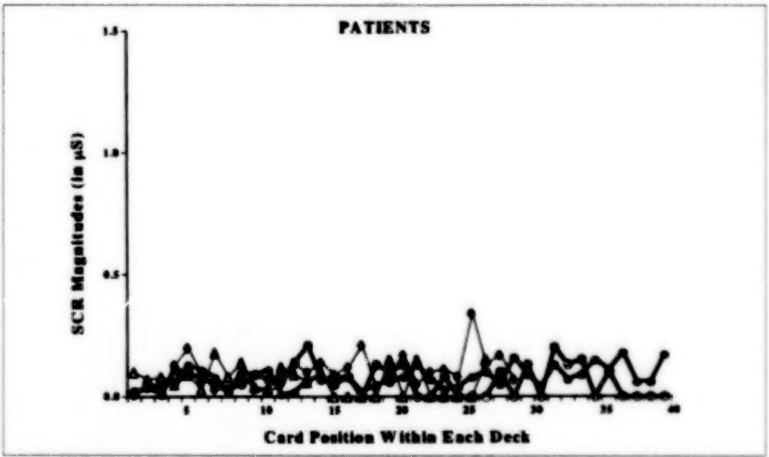

Figure 3. This graph is equivalent to the one shown in figure 2 but is obtained from the averaging of seven patients with damage to the frontal lobe. There is no buildup of skin conductance response (SCR) signal to the bad decks.

personal and social decisions that are adaptive and in their best interest. That is, they behave in a sociopathic way. This does not mean that they become criminals; they are different from developmental sociopaths, who are never able to reach appropriate decisions.

What mechanism, then, leads them to those wrong decisions despite their knowledge and intelligence? In part, these patients have lost the ability to change adaptively in new circumstances, to learn the kind of information that should guide their behavior. This is demonstrated in figure 3, which shows that unlike the normal individuals, patients with ventromedial frontal lobe lesions fail to acquire that covert nonconscious signal concerning the "goodness" and "badness" of the decks. As a result, their performance is profoundly abnormal.

This signal, an autonomic reaction, is a part of the set of responses we call an *emotion,* and thus a partial basis for what we call a *feeling*. Not only do the patients fail to have this autonomic response during the anticipatory period of the task, they also fail to exhibit emotion in most circumstances in which a given personal or social situation would trigger such a reaction in most other mortals.

In other words, the problem that these individuals have with decision-making well may be related to the following:

- an inability to change dynamically vis-à-vis the task situation
- a consequent lack of production of a class of responses that can signal such changes.

That class of responses—which we call *bioregulatory*—includes autonomic discharges as well as emotions as a whole.

This finding, along with related findings regarding both normal people and patients with damage in specific neural systems, is helping us construct a neurobiology of rationality at the level of large-scale systems. We know, for instance, that the amygdala is necessary for this system as are structures in the brain core—the hypothalamus and brain stem—and selected parts of the somatosensory system.

Most importantly, these findings show that reasoning and deciding cannot be conceived along the traditional rationalist view that separates reason from emotion. On the contrary, reasoning and deciding both require the processes of biologic regulation. Both Skin-Conductance-Responses waves and emotions such as fear are expressions of that biologic regulation without which no survival is possible. In other words, the most complex processes of the mind, reasoning and creativity, cannot be separated from biological processes.

This is an example of plasticity at the level of a large-scale neural system in humans. We have demonstrated how adaptation to circumstances in the environment, filtered by previous experience and a previous value system, lead to changes in function over time. Those changes can be related to a particular neural system, some components of which have been identified. The system is important for the understanding of personal and social behavior from a neurobiological perspective.

There are several implications of these findings. First, since biological regulation, in general, and emotion, in particular, can play a role in the process of reasoning and deciding, the trivialization of emotion to which children are subjected is probably deleterious both to their brains and behavior. Second, it suggests that when designing educational strategies for children, relying too much on logical programs may not be the correct approach. In other words, educational strategies may have to take into account the fact that emotion does play a role in decision-making.

From the point of view of health management and policy, these findings help us focus on systems of the human brain that are probably dysfunctional in a class of psychiatric patients known as developmental psychopaths or sociopaths. It may well be that these patients, though they do not have damage of the type found in the neurological patients to whom we alluded above, may have profound abnormalities in the systems we have identified, perhaps at the level of neurochemical transmission and modulation.

Suggested Readings

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Carla J. Shatz graduated from Radcliffe College in 1969 with a B.A. in chemistry. She was honored with a Marshall Scholarship to study at University College London, earning an M.Phil. in physiology in 1971. In 1976, she received her Ph.D. in neurobiology from Harvard Medical School, where she was appointed a Harvard Junior Fellow. She studied with Nobel laureates David Hubel and Torsten Wiesel. From 1976 to 1978, she held a postdoctoral position in the Harvard Medical School Department of Neuroscience.

In 1978, Dr. Shatz moved to Stanford University and began her studies of the development of the mammalian visual system as a member of the Department of Neurobiology; in 1989, she was named professor of neurobiology. Three years later, she moved her laboratory to the Department of Molecular and Cell Biology at the University of California–Berkeley, where she is Class of 1943 Professor of Neurobiology.

In her 1993 presentation at the Library of Congress, Dr. Shatz considered three fundamental questions that motivate the research in her laboratory:

- How is the brain wired up during development?
- How do nerve cells know where to grow their connections?
- To what extent is vision necessary for the visual system to develop normally?

She then described the sequence of developmental events that occur, during both prenatal and postnatal life, to give rise to the adult wiring of connections between the eye and the brain in the mammalian visual system. One significant conclusion: our studies of nerve cell signaling during development and the role of signaling in forming normal adult connections have direct relevance to the clinical topics of neurological birth defects and abnormal visual function.

HOW SPECIFIC CONNECTIONS FORM IN THE DEVELOPING BRAIN

Carla J. Shatz

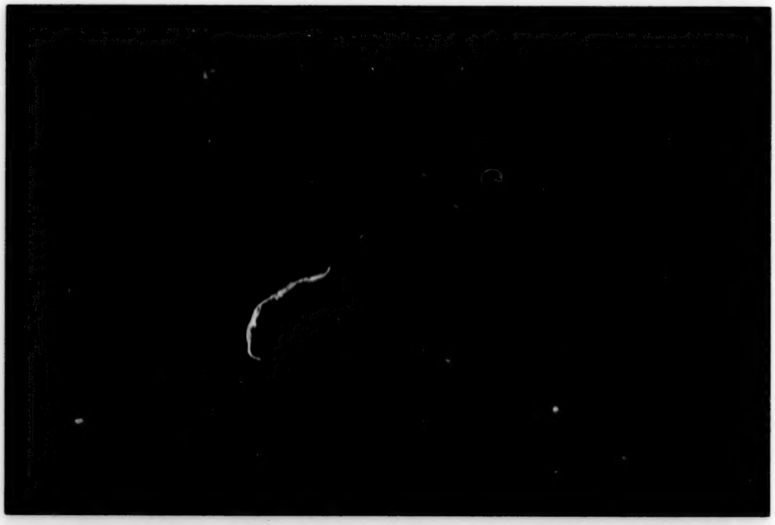
Individual brain cells respond to very specific visual stimuli. How can the brain early in development establish connections among its billions of neurons that result in such remarkable specificity for visual stimuli and other sensations?

The functioning of the adult brain is so precise that nothing, it seems, has been left to chance. David Hubel and Torsten Wiesel demonstrated in the 1960s, however, that there is a period soon after birth when chance can play a role in its development. The patterns of a young animal's experience influence how the brain develops. This influence is seen most dramatically in such abnormalities as cataracts or crossed eyes, both of which profoundly alter visual experience.

Many forms of neurological defect have no explanation. The brain looks normal, but it does not function correctly. The problem often lies in abnormal patterns of connections among the billions of nerve cells. Although some brain connections are formed in early childhood, a large proportion are established before birth. And some brain abnormalities, such as cerebral palsy, also are thought to occur before birth. It is essential, therefore, to understand how the brain establishes connections before, as well as after, birth.

A major question in studying the visual system is how the brain takes each of the two eye's separate views of the world and combines them into the single image we normally experience. Thanks to the pioneering studies of Hubel and Wiesel, this type of connection is well understood.

A set of nerve cells (composed of approximately one million neurons called *retinal ganglion cells*) extends connections from the eye to enter the brain at the relay station called the *lateral geniculate body*, as shown in figure 1. This relay station is the first place



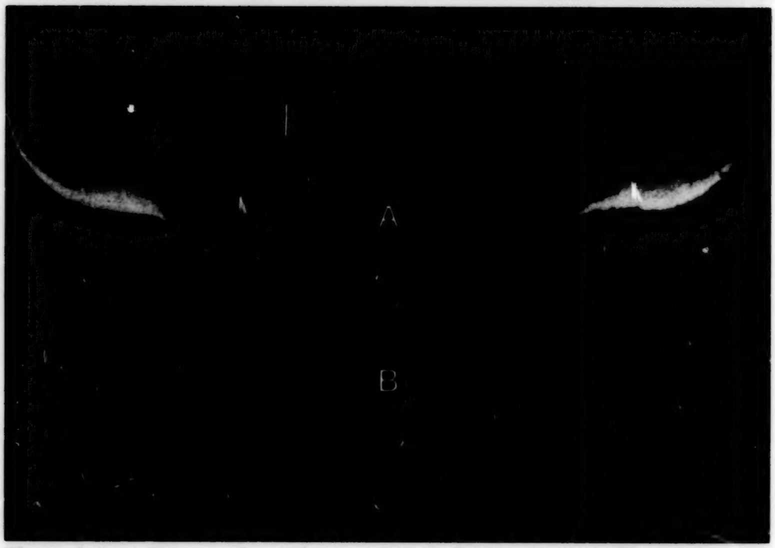


Figure 1 (*top*). Diagram of neural connections in the visual system. The output neurons of the eye, the retinal ganglion cells, send their connections to the lateral geniculate body, the first place where inputs from the two eyes meet. Inputs from the two eyes are not mixed with each other but rather stacked in a series of alternating eye-specific layers. The neurons of the lateral geniculate body, in turn, send their connections to the primary visual cortex in the back of the brain.

Figure 2 (*bottom*). Tracing the connections between the eye and lateral geniculate body using a nontoxic dye. The dye is injected into one eye and travels down all the connections from ganglion cells in that eye to their terminals within the geniculate; the dye appears bright white or pink and in the adult (A) is only present in the layers receiving input from the injected eye. Note that in the embryo (B), the dye is everywhere because layers have not yet formed.

where information received from the right eye and from the left eye come together.

Neuroscientists have demonstrated that in the lateral geniculate the inputs from the right and left eyes are not mixed together randomly; rather they show a strict order, which is typical of all brain organization. One aspect of that order is that the inputs from the eyes are grouped into a set of eye-specific layers that are stacked alternating the eye of input origin, as shown in figure 2. That is, a layer receiving input only from the left eye sits on top of a layer receiving input only from the right eye, which sits on top of a layer receiving input only from the left eye, and so on.

How does this kind of pattern—a stack of layers—evolve during development? It is helpful to envision the ganglion cells' long extensions, or processes, as phone lines extending between two cities. One eye might be Boston and the lateral geniculate the distant city of New York. Telephone wires must be connected between a million or so phones in each city without making erroneous connections to Providence or Albany. In the adult nervous system, unlike the telephone system, not every caller from Boston can reach every phone number in New York. Callers from the right eye can reach only locations in alternate lateral geniculate layers.

In the earliest stages of an animal's development, the eyes are not even connected to the brain. The sheet of cells that is going to form the nervous system rolls up into a tube on approximately the twenty-fifth day of human embryonic life; then the tube starts to bulge and individual parts of the system, including the eyes and the brain, begin to form. The retinal ganglion cells are created in the embryonic eye; the lateral geniculate cells are generated in a structure called the *diencephalon*, which is many cell-body diameters away from the eye.

Work by Corey Goodman and Thomas Jessel has demonstrated that the long outgrowths of a nerve, called *axons*, grow along the correct path and precisely select their correct target. The tips of growing axons sense specific molecules on the surfaces of cells located along the path, or molecules released from these cells. Once at the lateral geniculate, the ganglion cell axons array themselves so that cells located near one another in the retina contact neighboring cells in the lateral geniculate. The ganglion cells tend to follow the same route and end up grouped together in cables resembling the trunk lines of the telephone system.

The trunk lines of axons are formed early. In the human brain, the connections from the eye to the lateral geniculate nucleus are established by the end of the second trimester. The next set of visual system connections, however, from the lateral geniculate body to the primary visual cortex at the back of the brain (see figure 1), are formed only partially at birth. Thus there is a period of time after birth when visual experience can influence the formation of those connections.

Not only do connections have to form in *utero* from the eye to the lateral geniculate, but they have to link each eye with the appropriate layers. It is as if phone lines from one part of Boston must end up n New York's Upper East Side, whereas phone lines from another part of Boston end up on New York's Lower West Side. There is great precision in the eye-brain connections.

The Mechanisms of Development

There are several mechanisms whereby such precision might be achieved during development. Let's consider, for example, an analogy with computers. The precise connections within a computer depend on the manufacturer following a complex circuit diagram. One could conceive similarly of a biological blueprint: the genes would specify each connection between every nerve cell in the eye and every nerve cell in the lateral geniculate and, for that matter, among all the billions of nerve cells in the entire nervous system. The blueprint would include the connections from the ear into the part of the brain that is involved in hearing, from the fingers to the part of the brain that is involved with touch, and all the connections among the different areas within the brain. If this analogy were correct, the brain would wire itself during development by taking all of the various component parts, consulting the circuit diagram, and hooking them into the adult pattern. Then, at the figurative flip of a switch, the brain would begin to function.

Although the analogy with computers is appealing, many detailed studies examining brain development in animals indicate that biology does not work that way. Neuroscientists were greatly surprised to learn that the brain does not initially wire itself according to the adult circuit diagram. Brain activity, moreover, does not begin with the flipping of a switch. In fact, the brain must be active as the connections are forming; it must be functioning in order to wire itself.

This alternate image of brain development again derives from animal studies, because it is extremely difficult to study connections in the human brain. Neuroscientists have injected a tracer into one eye of fetal monkeys and cats and determined that the lateral geniculate layers are not present early in development. The first connections between eye and lateral geniculate make a diffuse pattern rather than forming layers. At the earliest stages, both eyes make connections all over the target structure. Later the connections are remodeled to remove the left-eye input to the right-eye layers and the right-eye input to the left-eye layers.

This modification of wiring during development is an important discovery. It has extensive clinical implications: brain wiring can go awry during development not only by abnormalities of nerve cells and failure of connections to form, but also by a failure of the remodeling process.

How does this remodeling occur? How does a brain determine which connections to maintain and which connections to remove? Again consider the analogy of phone lines in New York City. In development, wires from phones in all areas of Boston initially would be strung all over New York. One way of learning more about the phone connections would be to eavesdrop on some of the lines and determine who is talking to whom. Neuroscientists are using microelectrodes to eavesdrop to learn which fetal nerve cells are sending signals to which others.

This technique shows that the cells are sending signals to verify which sets of connections are appropriate and which sets are inappropriate. It is as if initially phone calls were being placed all over New York simultaneously, so that if you tried to call your grandmother who lives on the Upper East Side, the phone would ring in her apartment as well as in hundreds of others all over the city. With each call you make, however, some of the inappropriate connections would be removed, so you would get fewer and fewer wrong numbers. The fastest way to improve the phone system would be to use an automatic system to keep dialing your grandmother's number again and again.

In the brain, the equivalent to a phone call is the electrical signal sent along one ganglion cell from the eye to the lateral geniculate body. Does the nervous system really use some sort of automatic dialing in order to correct the original pattern of connections when it remodels? One way to answer that question experimentally is to stop the nerve cells' signaling by administering nontoxic drugs that block nerve cell activity.

David Sretavan, Michael Stryker, and I implanted in utero cat embryos with miniature pumps containing the drug tetrodotoxin just before the time at which lateral geniculate layers normally begin to form in the fetus. The drug temporarily blocked the flow of nerve signals from eye to brain and consequently the normal layers of the lateral geniculate failed to form. In an animal in which drugs stopped the signaling for two weeks, the connections from a single eye remain extended all over the geniculate area, rather than separating into layers of only left-eye or only right-eye connections.

This type of animal experiment has implications clinically for humans. In utero, drugs of abuse might block this dialing process; for instance, neuroactive drugs such as heroin or cocaine might interfere with the dialing and lead to the persistence of connections that normally would have been eliminated during development. In the phone system analogy, it would be as if the connections were established from one city to the other but the connections remained imprecise. Every time you try to call your grandmother, in that case, you call many other people as well.

How are the phone calls being placed? The remodeling of connections in the human lateral geniculate occurs before the end of the second trimester. Although some light can reach the fetus, the light-detecting cells—the rods and cones—are not yet present in the retina, so no actual vision is possible. The signals going from the eye to the brain, then, cannot be the result of light hitting photoreceptors. What seems to be happening is that there is automatic dialing occurring.

Figure 3. Six snapshots of nerve cell signaling during development. In each snapshot, the large black dots represent nerve cells in the eye that are sending signals to the geniculate at that moment; the small black dots are silent nerve cells. Each snapshot takes half a second. With each successive snapshot, different nerve cells are active, as if a wave of activity sweeps across the eye during the entire four-second recording period.

Neuroscientists are studying this auto-dialing process by removing small pieces of the fetal brain of mice, ferrets, and cats. The cells in this tissue can be kept active in a dish supplied with oxygen and other chemicals for ten to twelve hours, long enough for scientists to measure the signals that some of the nerve cells send to others. A new invention by Jerry Pine and his colleagues at the California Institute of Technology allows scientists to determine the activity of one hundred nerve cells at once. These new electrodes have been used to record all the nerve signals sent by one hundred of the retinal ganglion cells that carry information from the eye to the brain.

This technique, applied during fetal development, can provide a representational snapshot of how these nerve cells are sending signals. Are they firing randomly? The answer turns out to be far more interesting. Neighboring nerve cells in the eye fire at approximately the same time, the equivalent of a neighborhood phone-dialing frenzy; then cells in the next neighborhood become active. Eventually a wave of signaling sweeps across the retina, as shown in figure 3. After a wave of activity, the retina rests for about two minutes and then another wave is produced.

In the fetal eye in utero, these waves of activity occur over and over for weeks, but they are only present before the eyes begin to work. Once the eyes open, vision provides input to the cells, and these automatic waves disappear.

Cells That Fire Together Wire Together

How might the pattern of waves of activity help the brain remodel its connections? A recent theme in neuroscience is "cells that fire together wire together." Think again in terms of New York telephones. Consider two neighbors who place calls at the same time causing two phones elsewhere to ring simultaneously. The connection of each of these callers to the recipients would be strengthened. A caller in a different part of the city might also place a phone call at the same time, but no other neighboring phone would be ringing, so the connection would not be strengthened. The timing of signaling and the neighborhood properties of brain activity are essential for remodeling the early connections into the adult pattern.

Although the studies I have described focus on the visual system, the process is likely to occur in other areas of the brain as well. It is a general mechanism that could produce a precise pattern of connectivity in any brain area. There is already preliminary evidence of spontaneously generated signaling in the ear in utero before the fetus can respond to external sounds.

The fetal brain in development is not a miniature version of the adult brain. It is very different from the adult brain, but through a dynamic set of processes that require neural activity, the developing brain remodels itself to produce the adult pattern of connections. Some of the changes occur in utero and some soon after birth.

Why does the nervous system have such a complicated procedure for setting up and then remodeling the connections on the basis of automatic activity patterns? The likely answer is that there are not enough genes in the whole genome to give rise to the precision of connections that are present in the adult brain. In the visual cortex alone are more than a million connections. The brain uses the genome to set up a very basic framework of connectivity and then uses correlations in nerve cell activity to refine these connections into a final pattern.

A challenge for neuroscientists is to figure out the genetic rules that underlie the remodeling procedure. What information is present in the DNA, and what other molecules are required?

The clinical importance of the findings thus far is the realization that processes long before birth are crucial to the formation of normal connections in the brain. Anything that could disrupt this signaling between nerve cells could have profound implications for brain wiring and subsequent function of the brain after birth. Further studies might reveal how drugs of abuse can perturb the wiring of the nervous system and give rise to profound learning disabilities after birth.

Suggested Readings

Goodman, C. S., and T. M. Jessell, eds. 1992. Development. Special issue of *Current Opinion in Neurobiology* 2 (1) (February).

Shatz, C. J. 1990. Competitive interactions between retinal ganglion cells during prenatal development. *Journal of Neurobiology* 21 (1) (January):197–211.

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Shatz, C. J. 1992. The developing brain. *Scientific American* 267 (3) (September): 60–67.

Paula Tallal, professor of molecular and behavioral neuroscience, is codirector of the Center for Molecular and Behavioral Neuroscience at Rutgers, State University of New Jersey, where she has been on the faculty since 1988. She received her B.A. degree from New York University in 1969 and her Ph.D. from the Department of Experimental Psychology at Cambridge University in 1973. From 1974 to 1979, Dr. Tallal was on the faculty of the Johns Hopkins School of Medicine. In 1979, she joined the faculty of the University of California-San Diego, where, in 1986, she was named professor of psychiatry.

Dr. Tallal's presentation at the Library of Congress in 1993 described twenty years of studying rapid temporal processing deficits in language-impaired and reading-impaired children. Her research has demonstrated that slowed temporal processing directly interferes with adequate perception of those speech sounds characterized by very rapid acoustic changes that must be processed if speech is to be perceived accurately. These studies not only have broad implications for understanding the etiology of developmental language and reading disorders, but they also have raised fundamental questions pertaining to how the brain develops specialized speech processing in the left hemisphere. This research provides a new animal model for studying nonverbal temporal processing, which in turn enables scientists to investigate many basic aspects of the neurobiology of sensory processes that may be precursors to speech development. Dr. Tallal was the first to propose and demonstrate that a difficulty in understanding rapidly changing speech frequencies is the fundamental source of language problems in children with specific language learning impairment. She now collaborates with Dr. Michael Merzenich (see next chapter) in developing new training techniques to speed sound-processing and improve language skills in children with specific language impairment.

NEUROBIOLOGICAL AND NEUROPSYCHOLOGICAL STUDIES OF LANGUAGE DEVELOPMENT AND DISORDERS

Paula Tallal

Of the 67 million children in the United States, estimates are that approximately 10 percent have a language-based learning disability. There are many known causes of developmental speech delay or language delay (or both combined). Language delay can result, for example, from general mental retardation, hearing loss, motor impairment, or neurological disorders such as seizures or autism. There are also children who have none of these problems but fail to develop language at or near the expected age. Such developmental language impairment of unknown origin has come to be called *developmental dysphasia* or *specific language impairment* (LI).

Longitudinal research studies that have followed the development of LI children from their preschool through elementary school years have shown a striking relationship between early language impairments and subsequent academic achievement disorders, specifically reading impairments (developmental dyslexia). Thus, children with developmental language impairment are at exceptionally high risk for developing learning disabilities that potentially can lower their success and productivity throughout their lifetimes.

Given the high incidence of this serious developmental disorder and its negative impact on all other aspects of a child's development, one would expect that understanding the causes of this disorder would be of high priority to our nation. In fact, as these excerpts from a letter I received reveal, this has not been the case. This grandmother describes poignantly the current state of the art confronting most LI children.

> Tommy was born in 1986. . . . He developed normally in every way—but at age two we began to realize he had a language delay. He made crude sounds, but spoke barely an intelligible word. His pediatrician said not to worry. . . . But

> as time went along and "normal" speech did not develop, other professionals were consulted: nose, ear, throat specialist . . . pediatric neurologist. . . . So, at approximately two and a half Tommy was enrolled with his first speech professional—a nice lady with a master's degree. For whatever it's worth, he was labeled "dysphasic" and seems to be left-handed.
>
> At nearly six years old, Tommy is now working with his third "nice lady" with a master's degree and . . . the progress is so painfully slow. The truth is no one except five people—his mother, father, 8-year-old sister, current therapist and pre-school teacher (who was his first therapist)—can understand what he says. No, not even me, his grandmother. He is big for his age and looks 7 or 8. He turns heads at the mall with his unusual, strange, rhythmic, primitive sound pattern. It is very, very sad. He couldn't even tell someone his name and address if lost at the mall. This poor, handsome, precious little boy. What will become of him? Who will understand and be able to help him?
>
> . . . We have explored every lead. Every time we see something in print we write or call. We get so many trite remarks, such as, "Do you read to him? Maybe his sister talks for him. Maybe he doesn't have to talk. Maybe you should take him to Mayo Clinic" (or whatever famous medical center isn't in Oklahoma). Or, sometimes we receive pamphlets on the physiology of the brain. . . .
>
> When I taught kindergarten years ago, I used to read the children the story "Nobody Listens to Tommy." It was a favorite. And now the words of that title seem prophetic. A wonderful little boy wants to be heard.

There are millions of children like Tommy. The research in my laboratory is focused on understanding what causes their language problems. It is our perspective that until we do the research necessary to work out the underlying etiology of language and learning disorders, we will not have the foundation of knowledge critical for developing more directed and effective early diagnosis and treatment procedures. Fortunately, by using neuropsychological and neuroscience research approaches, we have made considerable progress in understanding the neurocognitive and neurobiological basis of developmental language and reading disorders.

The Role of Rapid Temporal Integration Mechanisms

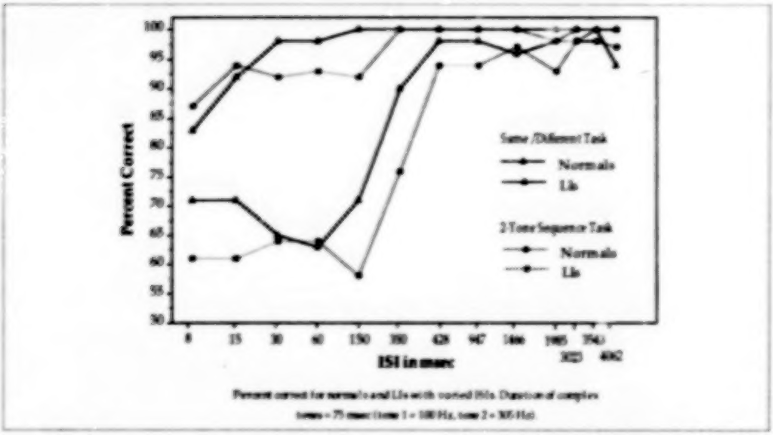
The primary tenet of my research is that higher cognitive functions are built upon more basic underlying neurobiological processes. If we are to bridge the gap between neurobiology and behavior, however, we must understand the fundamental components and mechanisms of cognitive processes at the behavioral level at least as well as we understand neurobiological processes at the molecular and cellular level.

I have focused my research on investigations of the neurobehavioral basis of speech and language processing. Because studying cognitive dysfunction provides a unique window through which more basic neurobehavioral mechanisms can be elucidated, I began my research by studying children with a severe developmental language impairment. Following the data derived from a systematic progression of studies over the past twenty years has led me to propose a global neurobiological theory centering on the preeminent role of rapid temporal integration mechanisms for speech processing, as well as left hemisphere specialization for language.

I began my research in 1970 as a graduate student in experimental psychology at the University of Cambridge. I was interested in understanding the neural basis of severe deficits both in phonological (speech) perception and production which characterized children with developmental language impairment. I reasoned that before studying language processing, it would be important for me to assess the integrity of the component acoustic processes critical to analyzing the complex acoustic wave form of speech. I developed a novel research paradigm to investigate systematically the critical stages of central auditory processing in young children.

Using this paradigm we found that LI children required significantly more processing time both to discriminate and to sequence auditory stimuli.[1] Subjects were operantly conditioned to detect, discriminate, and serially recall varied sequential presentations of discrete stimuli and to respond by pressing panels on a response box in the order corresponding to stimulus presentation. LI children and matched controls were tested with these procedures using two 75-millisecond steady-state, complex tones differing in fundamental frequency. In trials in which more than one tone was presented, the inter-stimulus-interval (ISI) was varied between 8 and 4,062 milliseconds.

Figure 1 demonstrates that there were no significant differences between the performance of LI and control children on a subtest when the ISI was 428 milliseconds or longer. The perfor-



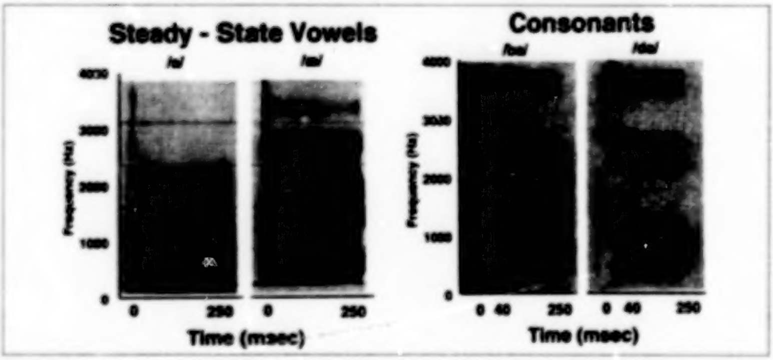


Figure 1 *(top)*. Language-impaired chidren require more processing time, measured in milliseconds, to discriminate and sequence auditory stimuli.

Figure 2 *(bottom)*. Examples of the acoustic wave form of two classes of speech sounds.

mance of the LI children, however, deteriorated rapidly with shorter ISIs. No LI subject reached a criteria of 75 percent correct at a 150 millisecond ISI or shorter. In contrast, all controls were able to reach 75 percent correct at ISIs of 8 milliseconds or longer.

Studies of Speech Perception and Production

Could such a basic temporal integration dysfunction undermine speech and language development? The results of the psychoacoustic studies pointed to temporal dysfunction within the tens-of-milliseconds range, the time frame within which temporal cues critical for phoneme (speech sound) perception occur.

Figure 2 shows examples of the acoustic wave form of two classes of speech sounds. As can be seen in these sound spectrographs (which display frequency changes that occur over time as speech sounds are produced), vowels transmit the same acoustic information throughout their acoustic spectra, and are thus steady-state. Stop-consonant syllables (such as /ba/ and /da/), however, have a transitional period between the release of the consonant and the initiation of the vowel during which the frequencies (called *formants*) change very rapidly over time (within about 40 milliseconds). Information carried within these brief formant transitions are critical for syllable discrimination.

We hypothesized that LI children would be particularly impaired in discriminating brief duration temporal cues within speech, such as the brief formant transitions within stop-consonant-vowel (CV) syllables, but would be unimpaired in discriminating between speech sounds that are characterized by steady-state or more slowly changing acoustic spectra, such as vowels. We tested this hypothesis by evaluating LI children's ability to discriminate speech sounds, which we synthesized on a computer to gain control over the temporal components. The results were dramatic. LI children were able to discriminate steady-state vowels but unable to discriminate CV syllables incorporating 40 millisecond duration formant transitions.[2]

We next sought to determine whether LI children's poor performance on tests with CV syllables could be improved by extending the duration of the formant transitions from 40 to 80 milliseconds. The results were exciting; they showed that the LIs were unimpaired in processing the same CV syllables they were previously unable to discriminate when the duration of the critical formant transitions were extended from 40 to 80 milliseconds.[3]

Additional studies testing subjects' ability to discriminate many different speech sounds based on a variety of temporal or spectral cues confirmed the hypothesis that LI children are selectively impaired in their ability to integrate brief acoustic components occurring within tens of milliseconds in the ongoing speech stream.[4] Spectrographic analyses of speech production data from LI and control children also showed a remarkable similarity between the pattern of temporal production impairments and temporal perception impairments.[5]

This remarkable mirroring of specific temporal constraints in both sensory and motor systems subserving speech has important implications for theories that pertain to neural mechanisms underlying speech in humans. We then aimed subsequent studies at determining whether the degree of deficit in nonverbal temporal integration would correlate with higher level aspects of linguistic processing beyond the phoneme level. When we used a comprehensive battery of language tests, we found a strikingly significant correlation ($r = .85$, $P < .001$) between the degree of temporal processing impairment and the overall degree of receptive language impairment.[6] These data demonstrate that a primary inability to process acoustic information entering the nervous system in rapid succession initially may disrupt the development of phonological processes and subsequently lead to more global language delay.

Modality Effects

We were interested in whether this pattern of temporal processing deficits occurred only in the auditory modality for both nonverbal and verbal stimuli or was a more general deficit that affected other sensory and motor systems. To test the modality specificity of these temporal deficits, we developed a comprehensive battery of nonverbal and verbal sensory and motor studies designed to investigate visual, somatosensory, and cross-modal sensory integration, as well as rapid sequential motor output. The results showed that LI children are profoundly impaired in their ability to discriminate, sequence, or remember any brief stimulus if followed in rapid succession (tens of milliseconds) by another stimulus, regardless of the modality of stimulation.

We found a similar pattern for the production of rapid, sequential oral or manual movements: deficits regardless of whether the stimuli were verbal or nonverbal.[7] The results of these studies demonstrate that LI children have a pervasive pansensory/motor deficit that is manifested in many aspects of their speech, cognition, and movement behavior.[8]

Neuroimaging Studies

As we know, the developing brain remains plastic and has considerable capacity for recovery of function if damaged in childhood. It is difficult, then, to account for the severe, long-term language deficits of LI children at the neural level. Indeed, it is a paradox that children with developmental LI have a poorer prognosis for language recovery than do children with acquired lesions to left hemisphere structures known to subserve language.

Although several theories have been developed to account for this paradox, noninvasive techniques only became available recently to test these hypotheses at the structural level in living children. The recent advent of in vivo neuroimaging techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET) opened a new era for studying the neurobiology of higher cortical functions such as speech, language, and reading as well as studying the brains of LI children at the structural level.

In collaboration with Terry Jernigan at the University of California-San Diego, we reported the results of the first study of neuromorphology in LI children using MRI, a study that yielded striking new insights into the neural structures underlying developmental language impairment. Using volumetric analyses, we found significant—but heretofore unsuspected—reduction in gray matter volume in subcortical structures (including striatum and thalamus), as well as bilateral reduction in cortical structures known to subserve language. In addition, aberrant patterns of cerebral lateralization in the brains of LI children were demonstrated in both prefrontal and parietal regions. These results suggest more widespread differences in the development of the brain in these children, including both subcortical and cortical areas as well as bilateral differences in the brain regions known to subserve auditory processing and language. Importantly, highly significant correlations were found between the extent of aberrant hemispheric asymmetry of these cortical regions and the degree of auditory temporal processing deficits, linking for the first time structural and functional deficits in developmental language disorder.[9]

One consistently supported tenet in neuropsychology is that speech is processed and produced preferentially by the left cerebral hemisphere. Support for this fundamental axiom derives both from studies of adults who have sustained selective brain damage leading to specific functional disorders and from studies designed to evaluate differences in information processing within and between the cerebral hemispheres in normal intact subjects.

What is the neurobiological basis of this specialization? Why is speech selectively processed and represented in the left hemisphere, and what are the evolutionary precursors to hemispheric specialization for speech? On the basis of our studies with LI children, we proposed that hemispheric lateralization for speech may derive from a more basic specialization for the analysis of rapidly changing, or transient, sensory/motor information.

The Importance of Temporal Disruptions

Damage to a human's left cerebral hemisphere usually results in aphasia. Whereas damage to the left hemisphere is associated with impaired phonological processing, damage to the right hemisphere has been associated with impairments in nonverbal acoustic analysis.

To investigate whether this dissociation is based on temporal or linguistic processing constraints, I collaborated with Freda Newcombe in studying a well-characterized group of men with acquired missile wounds to either their right or left hemisphere. We examined whether damage to the left or right cerebral hemisphere disrupts nonverbal rapid temporal processing. The results demonstrated that contrary to expectation, processing of rapidly changing nonverbal acoustic information is severely disrupted by brain damage in the left—and not the right—hemisphere in adults.[10]

Similarly, adult aphasics only showed deficits in discriminating between speech sounds which incorporated brief, rapidly changing temporal cues. Like LI children, they were completely unimpaired in discriminating other speech sounds of longer duration, speech sounds that were steady, or speech sounds with more slowly changing acoustic spectra. These results support the conclusion that what is selectively damaged by left hemisphere lesions involves mechanisms critical for processing brief or rapidly changing temporal cues, regardless of whether they are verbal or nonverbal. Thus, a disruption of rapid temporal processing may lead to the phonological disorders characterizing aphasics.

One technique used extensively to study hemispheric specialization in intact normal subjects is the dichotic listening paradigm. A myriad of studies have shown that when competing verbal information is presented simultaneously (dichotically) to the two ears, subjects more often respond correctly to the information presented to the right, as compared to the left, ear. This right-ear advantage has been hypothesized to result from the right ear having primary access, via contralateral pathways, to the left hemisphere. The preferential processing of speech information presented dichotically to the right ear, historically, has been used as strong evidence of left hemisphere specialization for speech perception. What quality of left hemisphere processing, then, produces more accurate perception of speech?

My student Joyce Schwartz and I hypothesized that the left hemisphere has specialized mechanisms for rapid sensory integration within tens of milliseconds. Since temporal components of speech must be analyzed within this critical temporal range, this would predict that speech should be processed preferentially by the left hemisphere. We hypothesized that the temporal constraints—not the requirement for linguistic processing—underlie this specialization.

To address this hypothesis, we prepared two sets of computer-generated stop-consonant-vowel (CV) speech syllables. One set was synthesized to contain the typical 40-millisecond duration formant transitions. The second set, however, was synthesized with transitions extended to 80 milliseconds. These two sets of CV syllables were used to test the ear preference of normal adult listeners in a dichotic listening paradigm. As expected, we found a significant right-ear advantage for the dichotically presented syllables with 40-millisecond duration formant transitions, thus replicating the results of numerous previous studies. The right-ear advantage, however, was reduced dramatically for the same syllables generated with transitions extended to 80 milliseconds.[11]

This critical result prompts a reevaluation of theories pertaining to hemispheric specialization for speech. These data demonstrate that the left hemisphere may be specialized primarily for processing rapidly changing temporal information, including (but not limited to) speech. These data also provide strong support for the hypothesis that even what normal listeners selectively process in their left hemisphere may relate more to temporal constraints than to phonological processes.

PET Studies

To investigate further this hypothesis, I initiated a collaboration with Marcus Raichle, Julie Petersen, and Julie Fiez at Washington University in St. Louis. We designed our collaborative studies to investigate neural aspects of speech and temporal perception in normal adults using the brain imaging technique called positron emission tomography (PET). PET studies allow us to image the location of change in metabolic activity that occurs in various brain regions when an individual is performing a specific mental task or behavior.

In our PET study, subjects listened to four sets of sounds that were designed to determine which areas of the brain were significantly activated during speech and non-speech acoustic processing. We used speech stimuli that either did (syllables, words) or did not (vowels) incorporate rapidly changing acoustic spectra. We also used nonverbal stimuli that incorporated temporal changes within the range that occur in speech, but did not have verbal meaning.

We found that significant decreases in metabolic activity occurred bilaterally for all four sets of stimuli in a number of regions in the parietal lobe. Activity increased in both the left and right temporal and frontal cortex. One area in the left frontal cortex (Brodmann 45) was particularly interesting. This area was near Broca's area, a frontal area where damage often leads to aphasia. This left frontal area was significantly activated by the sets of stimuli that incorporated rapid acoustic change (syllables, words, and brief tone sequences) but not by steady-state vowels.

Importantly, significant distinctions in activation in the left hemisphere did not occur along verbal versus nonverbal lines but rather along temporal lines (rapid versus non-rapid frequency changes over time). These PET data from normal adults are consistent with the results of our MRI studies in which LI children failed to show expected cerebral asymmetry in the frontal and parietal regions. Again: the degree of aberrant cerebral asymmetry in these two brain regions was highly correlated with deficits in processing rapidly presented tone sequences. It has been thought that humans may have developed a module in the brain that is specialized only for speech, and different brain modules process non-speech acoustic signals.[12] These PET data fail to support this view. Clearly the same brain regions process rapid nonverbal and verbal acoustic stimuli. No specialized activation just for speech was found.[13]

Development of an Animal Model

Even though the basic mechanisms for encoding speech stimuli are technically a form of acoustic information processing, many investigators adhere to the notion that speech processing is "special," distinct from other acoustic processes, and only found in humans. This philosophy unfortunately has impeded the development of animal models to study the neurobiological basis of processes fundamental to speech perception and production. As a result, we know far less about complex acoustic processing and speech at a neurobiological level than we do about other higher cortical functions such as visual perception or memory.

Accumulated data from research, however, now provides substantial evidence that a common mechanism underlies the discrimination of verbal and nonverbal acoustic events that are characterized by rapid temporal change. Given these results, one can introduce the evolutionary hypothesis that auditory temporal processing represents a "precursor" to speech processing and, further, that left hemisphere specialization for this basic process occurs in other species.

Together with Holly Fitch, we have recently designed a series of studies to investigate the potential origins of left hemisphere specialization using a novel animal model. In these studies, adult rats were trained in a modified operant conditioning procedure that culminated in a test paradigm similar to a human dichotic listening test. Sequences of two tones separated by a brief interval were presented selectively to either the right or left ear, while white noise was presented to the opposite ear. Rats were trained to identify one "target" sequence (for example, a low tone followed by a high tone) out of three other possible negative (non-target) sequences.

The results demonstrated that adult male rats were significantly better at discriminating tone sequences with the right as compared to the left ear.[14] Like humans, then, the rats showed a significant right-ear advantage (REA), indicating left hemisphere dominance for rapid temporal processing. Human dichotic listening tests and tests of language recovery after left-hemisphere damage, furthermore, have shown a gender difference, which suggests stronger left hemisphere specialization of language function in males. When the performance of male and female rats was compared, results showed that male rats, like human males, were in fact significantly more lateralized to the left hemisphere than females.

These results are important for two reasons. First, they demonstrate that rapid temporal processing may play a central role in the evolution of hemisphere lateralization by demonstrating that lateralized mechanisms which ultimately may subserve speech perception in humans can be found in other species. In addition to evidence derived from studies with LI children that argues that basic temporal processing abilities may be a prerequisite for normal speech and language development, these animal studies showing left hemispheric lateralization in rats suggest that rapid temporal integration processes may underlie, at least in part, the evolution of speech processing mechanisms.

Second, the development of an animal model to study the temporal processes impaired in language-impaired children provides a new opportunity to investigate central nervous system organization of a function that now appears to be a precursor to speech perception and production. We are currently focusing on neuroanatomical, neurochemical, and neurophysiological studies using this new rat model in an attempt to understand the neurobiological underpinnings of this important cognitive function. These studies hold exciting promise for providing new insights into the neural basis of language and reading disorders. They also have the potential to lead to completely novel early detection and remediation strategies for language-based learning impairments in children.

The long-term goal of this research program is to shed light not only on key issues underlying the mystery of developmental language and reading disorders, but also on fundamental questions pertaining to the neurobiological basis of phonological systems in humans and the origins of hemispheric specialization for language. The results of this research already have played an important role in changing some fundamental theories about the origins and bases of developmental language and reading disorders, as well as the evolution and neurobiological foundations of speech, language, and hemispheric specialization. Thus this research has important clinical as well as theoretical implications that have the potential to change significantly the future course of research on the neurobiological basis of speech and language, as well as the treatment of developmental language and reading disorders.

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Suggested Readings

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Hotline Numbers

For information about developmental language and reading impairments:

- American Speech, Hearing, and Language Association, **800 638-8255**
- National Dyslexia Research Foundation, **800 824-READ**
- National Institute on Deafness and Other Communication Disorders, **301 402-6251**
- National Institute of Child Health and Human Development, **301 496-6591**
- Orton Dyslexia Society, **800 222-3123**

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Dr. Merzenich studies changes in sensory systems as a way to learn more about the brain's "plasticity," its potential to establish new networks of connections between nerve cells in support of a variety of brain functions. His primary research interests are in cortical network physiology; cortical contributions to learning and memory; central auditory and somatosensory neuroscience; cortical contributions to the origins of stroke and bases of recovery from stroke; the origins and remediation of learning disabilities; and the further development of electrical stimulation prosthetic devices designed for hearing restoration for the profoundly deaf. He is the coinventor of a commercially applied cochlear implant.

The work Dr. Merzenich described in 1995 at the Library of Congress focused on the basic expressions of learning-induced changes in the brain that account for skill learning and perceptual learning. That research now is being incorporated into the design of new training programs that speed sound-processing and improve language-learning skills in language-impaired children in collaboration with Dr. Paula Tallal (see preceding article).

BRAIN PLASTICITY ORIGINS OF HUMAN ABILITIES AND DISABILITIES

Michael M. Merzenich and William M. Jenkins

Over the past fifteen years, there has been a sea change in the way in which neuroscientists view how learning and memory originate in cerebral cortex mechanisms. Our field of interest is studying how each of us evolves specific individual behavioral capacities based on this self-organization of the brain.

Historically, the cerebral cortical mantle that encompasses about half the volume of the human brain was most commonly viewed as a special computer with fixed elements and interconnections. That view was fueled in part by earlier studies demonstrating that cerebral cortex neurons developed adult-like morphologies and response properties and came to be distributed in adult-like forms in the visual and somatosensory cortex through a "critical period of development" early in life.

More recent studies have led to a radical revision of this view. We now understand that cortical neuronal responses and local brain circuits are continually modified by our experiences. Even our most precise and reliable and stable cortical representations of the surfaces of the skin, of the sounds in hearing, or of our most elementary movements are remodeled by any new, cognitively important experience. The brain literally remodels itself in detail throughout life.

We first documented this cortical dynamism in the cerebral cortex of adult monkeys, in which we tracked changes in orderly brain representations of the skin surfaces that followed the elimination of inputs from a significant sector of the skin, for example, from one or two fingers.[1] According to the earlier view of an anatomically static forebrain, the cortical regions representing these skin surfaces should have remained silent after such manipulations. In fact, they were quickly occupied by progressively topographically expanded representations of the surrounding skin.

Dramatic representational remodeling occurred even when the skin inputs were not altered, for example, when two fingers were fused surgically without the nerves innervating the skin being disturbed; it happened when two long-fused human fingers were separated; and it happened when an always-innervated island of skin was transferred across the hand in a plastic surgery "skin-island-transfer" procedure.[2]

The nature of the representational changes recorded in these studies supports a very large body of evidence that demonstrates that cortical change mechanisms are coincident-input dependent.[3] That is, the way a hand is mapped out in the cortex before and after these procedures is consistent with the hypothesis that cortical representational topographies are governed by the topography of temporal inputs. When the schedules and sources of nearly simultaneous inputs from the skin are altered, cortical maps of the skin change correspondingly, following these rules:

- Activity delivered simultaneously in time is integrated (represented together) by cortical neurons.
- Inputs consistently delivered almost simultaneously are represented as neighbors.

Considered in detail, cortical representations are time-based constructs, and they can be modified at any point in life (with certain behavioral contingencies described below) by changing the quantities and the temporal distributions of inputs.

Cortical remodeling of body surface representations apparently can result in an occupation of very large cortical zones deprived of their primary initially effective inputs.[4] Maps of skin responses or of movements, for example, reveal that the entire zone formerly representing the skin and the movement of an arm can come to be occupied by an expanded representation of the proximal arm stump or (in a smaller group of patients) face skin, or by an expanded representation of the movements of proximal limb muscles.[5] In these examples, cortical zones that are several square centimeters in extent, when deprived of their principal effective inputs, appear to be occupied by new and expanded representations of surrounding skin only a few weeks to months after the initial injury.

Cortical Plasticity Underlying Learning

The representational remodeling following alterations of peripheral injury induced by surgical procedures and by peripheral nerve and brain injuries actually reflect the operation of normal learning mechanisms introduced by the dramatic alteration of effective sources of information delivered into the brain in these special peripheral-injury or brain-injury circumstances.

Learning-based cortical plasticity has been the subject of intense study over the past decade. Many studies have shown that the responses of neurons in wide cortical zones that represent the conditioned stimulus (for example, a puff of air delivered to the tip of the nose), the unconditioned stimulus (for instance, a tone), and the conditioned response (for example, a tone-induced twitch or flinch) are altered to represent them in an amplified, highly selective way.

In adult mammals, the specific responses of hundreds of thousands of neurons and many hundreds of millions of synapses are formed or altered in the cerebral cortex to accomplish this change. Many more neurons represent these specific behavioral stimuli and behavioral responses after conditioning than did before conditioning. Neurons responding to these events have heightened sensitivities and response selectivities. Neurons in zones representing these now-special stimulus and response events are progressively more strongly interconnected, and engaged neuronal populations acting cooperatively generate progressively more reliable and more temporally coherent responses. And the engagement of neurons is directly contingent upon the event sequence that occurs in the behavior.

When the behavior is "extinguished" by a period of consistent, random, nonassociated stimulus and punishment-or-reward events, all these plasticity changes fade from the cortex. Otherwise, they could endure for many weeks or months or, in some cases, for a lifetime. Moreover, if we "train" a mammal with consistently nonassociated inputs from the outset, a series of negative response changes for the unconditioned stimulus are recorded; that is, neurons come to be less sensitive than normal in responding to it, and respond less selectively than normal. Neuroscientists have elaborated beautifully the fundamental cellular, synaptic, and molecular changes that could underlie both the positive and the negative aspects of these changes.

We have studied these changes induced by learning in our own laboratory in another important way. Monkeys were trained in the development of new motor or tactual or auditory skills. They learned to make finer and finer distinctions between stimuli, to develop progressively more adept movements with practice, to sort complex stimuli more and more reliably, to improve at recognizing specific spectrotemporally complex target stimuli, and so forth.

We then tracked the changes induced by such progressive learning by "mapping" neuronal responses in a very fine grain all across the engaged zones of cortical areas in which these stimuli or movements are represented. Our objective was to define how neuronal responses and interconnections in the directly engaged neuron network sectors were altered, and how those changes might account for improvements in the monkey's behavioral performance.

Again, massive changes in neuronal responses were recorded in all of these experiments. Neurons over large cortical zones came to respond very selectively to stimuli applied in the learned behavior, representing the practiced part of critical stimulus continua, with progressively greater precision and finer detail. Neurons also represented these important stimuli with greater sensitivity. Neurons distributed across the engaged sector responded to them progressively more simultaneously in time, thereby representing them in a progressively more powerful synchronous-response form for the simultaneous input-based plasticity mechanisms of the brain, as the engaged neuronal syncytium came to be more and more strongly positively interconnected.

The scale of these changes in adult monkeys and humans is great. When we began to experiment on monkey models to derive changes in the brain and learning, we found that when an animal takes up any new enterprise, the result is rapid and dramatic remodeling of the representations of the body surfaces or movements, or of sounds in the hearing domain, that paralleled learning.

We trained a monkey to pick food pellets out of a series of food wells under conditions that ensured that initially the monkey would be successful in his retrieval only every tenth or fifteenth or twentieth time. When a monkey first performs this task, he may need to try ten or fifteen or twenty times for each successful retrieval; two or three weeks later, however, he will perform this behavior without mistakes. Initially his behavior is highly variable trial by trial, and he tries several different strategies. Ultimately, however, he performs this behavior in a highly stereotyped, beautifully repeated way that underlies his success.

To accomplish this task, the monkey must palpate and grasp the pellet with specific fingertip surfaces. When we look in the brain, we discover that as a consequence of one or two or three or four hours of practicing this movement, the specific skin surfaces used by the monkey are enlarged in representation about twofold; within these zones, the skin is represented in exquisite topographic detail, much finer than normal.[6] The fingertips the monkey is enploying in this simple task are not functionally the same ones the monkey started with a few hours before!

When we explore a cortical zone representing feedback information from muscles, we discover that there is a great exaggeration of just those inputs that are crucial for combinative movements that are involved in initiating important phases in the fluid movement sequence. When we explore a cortical zone representing movements themselves, we find a striking exaggeration of specific movements and movement combinations that are crucial for individual elements of the reach-palpate-grasp retrieval sequence.[7]

When others have explored premotor areas in which movement planning or the sensory guidance of movements are controlled, they have found that a large percentage of neurons in these zones are altered to represent the specific contingent conditions of the behavior. From even a simple behavior like this one—the kind we all initiate repeatedly—result massive distributed changes in brain representations that involve alterations in the selective responses of cortical neurons in sometimes twenty or more cortical zones.

Language-based Learning Deficits

What is the meaning of such plasticity? First and foremost, it means we should rethink how we view learning and its contributions to our individual lives.

Brain self-organizing processes operate powerfully to shape new learning throughout our lives. They must be considered as part of any enduring functional disability or chronic brain illness. Moreover, brain self-organizing processes themselves must be a source of functional disability.

One example relates to speech and language deficits in learning disabled children. A child is identified at the time she or he enters school as "learning disabled." The child's speech reception capabilities, language comprehension, and understanding of grammar may be more than two years behind a normal child of the same age. The child has a deficit in his or her ability to process sound inputs in time and cannot identify the sequence order of fast sound signals, which occur frequently in speech. Like a foreign-language learner who has not yet mastered a second language, the child cannot reliably recognize fast consonant elements in running speech. The child does not understand—and therefore cannot follow—simple instructions given by a parent or teacher; thus the child may be labeled as having an "attention deficit disorder." The child's speech and language deficits will cause him or her initially to fail at reading; to be slower in the pace of cognitive development than other children; and without carefully constructed family and professional support, to suffer severe self-image trauma that accompany the label of "failure."

How has this panoply of speech or language problems arisen in the brain of the child? What is actually wrong in the brain? Conventional studies tell us that inheritable factors may have played a role in the genesis of this problem.[8] At the same time, our child may have been put at risk by premature birth, or by an early history of severe middle ear infections, which are not necessarily paralleled by inheritable brain defects.[9] That is, inherited brain defects would not appear to be an essential determinant of this condition. An examination of the brains of such children reveals that they are both physically and functionally different from the brains of normal children, supporting the hypothesis that the inheritable factors may have affected brain structure and learning processes.

Before we blame the problem on physically expressed inheritable factors, however, we should remember that in the previous five or six years our child's brain has undergone an incredible self-organization in its attempt to create speech and language representations. In this struggle to produce a reliable representation of the child's native language, millions of phonetic elements have been produced and millions received. Syntax, grammar, and a growing lexicon have been created by this massive self-organizing process. Many cognitive aspects of language have been developed.

How has this powerful learning contributed to the expression of the learning disability recorded in our child? How much of the deficit could be a product of brain self-organizing processes struggling to create a salient representation of speech and language by use of a marginally successful strategy, a process that ultimately creates and embeds defective language constructs?

The answers to these questions are important for guiding rehabilitation strategies for at least two reasons. First, to the extent that the expressions of the disability are attributed to cortical learning-induced plasticity, they potentially can be remediated at a later age by learning. Although the inherited or acquired physical or chemical defect (if one exists in our child) may not be reparable, we potentially can "repair" an earlier history of the child's learning that has resulted in defective speech or language capabilities.

Second, to the extent that the expressions of these abnormal brain operations spring from normal brain self-organizing or learning mechanisms, they only can be overcome by learning. In that event, the defective speech or language performance of our child has been established by hundreds of thousands—possibly millions—of "learning trials." No pill, no once-a-week training, no overnight remediation can be expected to have much of an impact on the powerful brain self-organization that has created and then heavily reinforced defective speech or language constructs.

It might be noted that the brains of these children in whom speech or language capabilities appear to be impaired in so many ways simply cannot be physically identical to those of normal children because brain self-organizing processes generate major changes in local brain circuits and functionalities. Experience that is different on this scale is equatable with brains that are different in detail on a large scale.

At the same time, to the extent that apparent physical differences are effects of defective brain self-organization, some of these functional and physical differences well may be reversible by appropriate training in later life. It is important to note that the presence of functional differences in our young student does not mean that his learning disability necessarily arises from fundamentally defective brain machinery.

Our hypothetical child represents more than half a million children entering American schools every year. Studies in monkeys show us how the panoply of defects in such children might arise by learning in developing children. They led us to ask: If we establish a remediation training strategy based on our hypothesis, our understanding about cortical plasticity underlying learning, and our understanding about the basic defective information processing in these special children, can we significantly remediate the behavioral manifestations of learning disabilities?

In collaboration with Paula Tallal, we have accomplished just that. Two training strategies were developed on the basis of studies of brain plasticity in monkeys and descriptions of the temporal processing deficits in dysphasic and dyslexic children.[10] Those strategies then were applied in six- to nine-year-old speech-based or language-based learning disabled children in a special school setting.

First we created computer-mounted "games" to improve adaptively the defective temporal processing abilities of these children.[11] The objective of one game was to improve children's fast-sound sequence reception abilities; the objective of a second was to improve fast-phonetic element identification. Through these age-appropriate "games," children were driven to achieve progressively higher task performances on successive training days. With a total of seven to twelve hours of training at games played for at least twenty minutes each day for twenty training days, all seven children improved substantially at both of these tasks.

For fast-sound sequence reception behaviors, a hallmark of these learning-disabled children, average gains were about fifteen-fold, and performances of about half of eighteen trained children approached the normal after training. For the fast-phonetic element recognition task, again, every child improved substantially, ultimately identifying correctly target elements presented at normal fast-speech rates.

A second strategy was to train children in conventional speech and language exercises but with the use of speech that was synthetically modified so that fast-speech elements were intelligible for these children while strengthening their form of representation for driving learning-based changes in their brains.[12] Children were given speech therapy exercises using speech in this synthetic form for about two classroom hours per day. They also were given "homework," age-appropriate book tapes and educational CD-ROMs, all with modified speech tracks. Over this four-week period, speech therapist and teacher interactions with these children were largely limited to nonverbal communication.

The combination of these two forms of exercises resulted in dramatic gains in phonemic identification, grammar, and language comprehension in these children. Children improved, on average, by about two years in age-equivalent performance with only twenty days of training—about sixty classroom hours plus homework.

The fact that we can reduce so substantially the striking temporal processing deficit that marks these children's defective speech reception and can produce these major gains in phonetic element recognition, grammar, and language comprehension strongly supports the two hypotheses on which we based our trials:

- Much of the expression of this powerful, multifaceted disability arises from earlier learning; that is, the disability is a consequence of the brain organizing itself to load its language machinery with crucial experiential information using a defective learning strategy, or operating on initially defective sensory information.
- Using a strategy based on our understanding of the defective temporal processing in these children and the basic self-organizing mechanisms that operate in our brains in learning, we can remediate and possibly eliminate this condition in a large proportion of the children who suffer it.

Occupationally Based Movement Disorders

A second class of disabilities that have their origins in brain self-organizing processes are occupationally based movement disorders. A musician practices finger control on the violin for several hours each day. Within a very short period of practice time, movement control begins to disintegrate; despite intensive practice as a person attempts to reestablish control, digital dexterity further degrades. Whenever the hand is positioned in the normal playing posture and the fingers brought down on the strings, the hand moves reflexively with uncontrolled and unwanted movements. Movement between the affected fingers in this posture becomes difficult, although in every other respect the movement control of the hand may be normal. In time, the loss of movement control spreads in the hand, and generalizes to other movements and postures, now affecting the use of pens or forks, now injecting clumsiness and discomfort into everyday life.

This scenario occurs in different forms to hundreds of thousands of American workers each year—typists, musicians, accountants, and tool- and computer-users. Because pain and inflammation often accompany these repetitive strain injuries, many clinicians have believed them to be generated by movement-induced microtrauma in the hand or arm and treated them as such.

Our studies show, however, that massive learning-induced changes in the brain parallel the emergence of this condition and almost certainly account for the loss of movement control recorded in these subjects.[13] In this case, cortical self-organizing mechanisms underlying learning create a "trap" that leads to deeply embedded dysfunction.

How did our violinist fall into this trap? As noted earlier, cortical changes in learning are coincident (simultaneous) and input-driven. When sensory feedback from the hand arrives from different sources (for example, from two adjacent fingers) nearly simultaneously and repetitively in a learning exercise, the learning mechanisms in the brain integrate it.

Monkey studies, including experiments in which we have generated this syndrome by training, have shown us that four conditions must be met to degrade hand movement control:

- Nearly simultaneous inputs are produced from more than one finger.
- Those inputs must be highly stereotyped; if they vary substantially from one practice event to the next, the cortex creates

more—not less—refined representations of afferent feedback information and movements.

- These stereotyped movements must be repeated with multiple practice trials commonly extending over successive days or weeks because the induction of these changes by learning requires input repetition.
- These nearly simultaneous, stereotyped, repetitive inputs must arise from an attended behavior; "automatic" performances of skilled movements do not engage the learning machinery of the cortex. Only attended, immediately cognitively important inputs can generate these destructive representational changes.

When we apply these four conditions to a monkey, it may develop a "tendonitis" or a "focal dystonia" marked by a loss of control of digital movement. When we examine the brain's representations of sensory feedback from the hand, we find it to be grossly degraded by the "on-the-job" training it has undergone. It is easy to generate a degradation of the representation of individual fingers by such training. When tendonitis or focal dystonia emerge, it is commonly paralleled by an integration of the representations of adjacent fingers.

In the normal case, skin surfaces and muscle afferent and joint information from individual digits are clearly differentiated in a monkey or human. In our repetitive strain injury models, finger representations are fused and sensory responses are massively de-differentiated. In these cases, the brain has integrated formerly separate finger movements into a single, compound, multiple-finger movement. For our violinist, the control of two adjacent fingers has merged. When she initiates a fine movement of either digit, the resulting compound movement is generated. Simultaneous feedback information is generated by the compound movement each time it is produced by commanding fine differentiated movements, further strengthening its representation.

Now that we understand that great de-differentiating changes occur in the brains of these patients, we need to revise our thinking about how to repair this movement disorder. These powerful brain changes induced by learning only can be overcome by learning. Whatever the extent of the actual physical problem in the arm or hand, true recovery only can be achieved by restoring the elaborately differentiated brain representations that control hand and arm function. We have devised a therapy strategy based on this premise and have achieved outstanding initial success in treating these patients.

Some Implications for Neurorehabilitation

Contemporary neuroscience has provided us with an increasingly deeper understanding of brain dynamics underlying learning. We have an increasingly clear vision of how we can use that perspective to define what brain self-organizing processes contribute to human dysfunction and disability. Understanding brain self-organizing processes in detail represents the crucial first step for applying them intelligently for rehabilitative good. Initial clinical trials employing this new information and perspective have created powerful new rehabilitative strategies that promise possibilities of remediating a wide variety of developmental and acquired functional disabilities in humankind.

Both language-based learning deficits and occupational movement disorders are produced directly by the progress of human culture. Repetitive strain injuries arise from heavy repetitive tool use that rarely occurred in earlier human societies. Dyslexia is a product of an evolving requirement for high-speed speech and reading, both inventions of the last millennium or two.

Brain self-organization has limits. The complex evolution of learned constructs that is so much a part of what each of us is cannot be expected to occur flawlessly always, without error. As our culture evolves and we exploit powerful ways to deliver massive amounts of information into our nervous systems at high speed, a deeper appreciation of how our interaction with our environment drives changes in our brains—for good and for ill—will increase in importance.

The basic research described in this brief review was conducted with the support of grant NS–10414 from the National Institute of Neurological Diseases and Stroke; by a series of contracts from the Neuroprosthesis Contract Program in the Fundamental Neurosciences Division of the Neurological Institute; and by Hearing Research, Inc. Learning disability remediation research was supported by the Charles A. Dana Foundation and by Hearing Research, Inc.

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Dr. Nicoll's research has focused on the mechanisms involved in synaptic transmission in the central nervous system. One of the first investigators to exploit the many advantages of in vitro slice preparations of the central nervous system, he demonstrated the role of the simple amino acid GABA as a neurotransmitter at many sites therein. Consequently, he was able to show that general anesthetics enhance GABA-ergic synapses, an action now recognized as playing a key role in general anesthesia. He also has contributed to understanding the signaling role of neuropeptides in the central nervous system. For a number of years, Dr. Nicoll's research has focused on the mechanisms involved in long-term synaptic plasticity. He has studied the role of Ca++ and Ca++ dependent kinases in synaptic plasticity and found that—depending on the form of plasticity—these kinases can either increase the sensitivity of the postsynaptic cell to the released neurotransmitter or enhance the amount of neurotransmitter released.

Speaking at the Library of Congress in 1995, Dr. Nicoll described his research into the mechanism by which the brain stores memories by changing the strength of synaptic contacts. He presented evidence that this change in synaptic strength involves a change in the sensitivity of the synapses to the chemical messengers. Such knowledge, he concluded, may provide the key for designing clinically useful therapeutic agents.

IN SEARCH OF THE MOLECULES OF MEMORY

Roger A. Nicoll

Understanding what changes in our brains as we are learning and remembering remains the most fascinating unsolved problem in neuroscience. We seek to understand what these changes are and where in the brain they occur.

We first gained some insight into these issues in the early 1950s, when a patient, who is now referred to as H.M., underwent a neurosurgical procedure to relieve intractable epilepsy. The surgery involved removing bilaterally a small area of the brain called the hippocampus. Although this surgery cured H.M.'s epilepsy, it left him—much to everyone's surprise—with a profound memory deficit. He was able to recall memories from his deep past, but he was completely incapable of forming new memories. You might have a seemingly normal conversation with this patient, for example, but if you were to leave the room and then come back, he would not remember that conversation ever had taken place. You would be an utter stranger to this patient, and you forever would remain a stranger.

A great deal of work on amnesiac patients involving neuroanatomical studies as well as imaging studies with NMR (nuclear magnetic resonance) and PET (positron emission tomography) have confirmed that the hippocampus plays a key role in memory.

What occurs in the hippocampus that is responsible for the laying down of new memories?

It is well established that when we engage in learning and memory, the changes that occur in our brains are stored as changes in the strength of the contact between nerve cells, the synapses. The experimental approach that many investigators use is an extremely reductionist one. By taking apart the brain right down to its individual molecules, we can get deep insight into complex behavioral problems such as learning and memory. With this knowledge

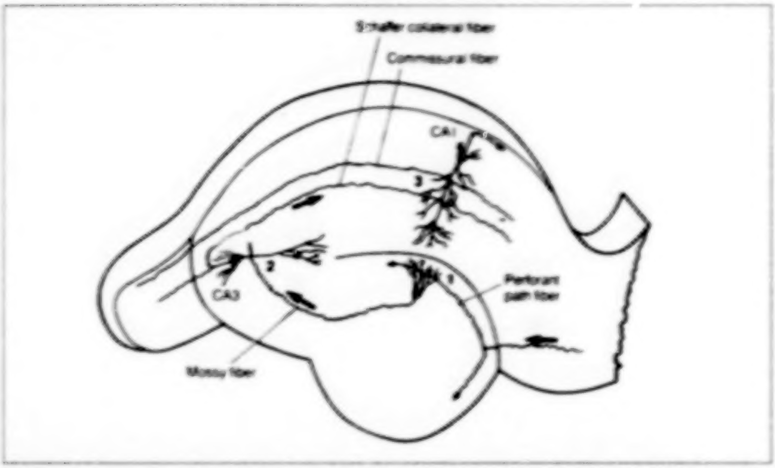

Figure 1. Diagram of the hippocampus showing the major excitatory pathways. All of the pathways exhibit long-term potentiation (LTP). CA1 and CA3 denote the pyramidal cell fields of the hippocampus; the arrows show the direction of impulse flow through the hippocampus. (From R. A. Nicoll, J. A. Kauer, and R. C. Malenka. 1988. The current excitement in long-term potentiation. *Neuron* 1:97–103. Copyright © 1994 by Cell Press. Used with permission.)

we can develop strategies that can be taken back to the complex behavior, to the intact animal, and to the clinical setting.

After we deeply anesthetize a rat, a commonly used animal in these studies, we quickly remove its brain from its skull. We next remove its hippocampus, cut that into thin slices, and put these slices into a dish that contains an oxygen-enriched solution. Figure 1 shows a diagram of the hippocampal slice and the neuronal circuits present in the slice. Only a few cells are shown, but millions of nerve cells exist within a slice. These nerve cells have branches, or axons, that make synaptic contact with other cells.

These synapses can be artificially activated by electrically stimulating the axons with an electrode, and the consequences of activating these fibers can be recorded within the nerve cells. Our ability to isolate the hippocampus allows us to perform a great many manipulations that otherwise would be impossible, and it permits us to dissect out the individual processes involved in synaptic transmission.

The synapse is composed of two parts: a presynaptic terminal and a postsynaptic density. The presynaptic terminal contains many round uniform vesicles which, at excitatory synapses, release glutamate. When an impulse or signal arrives at the terminal, one or more of these packets fuses with the terminal membrane and

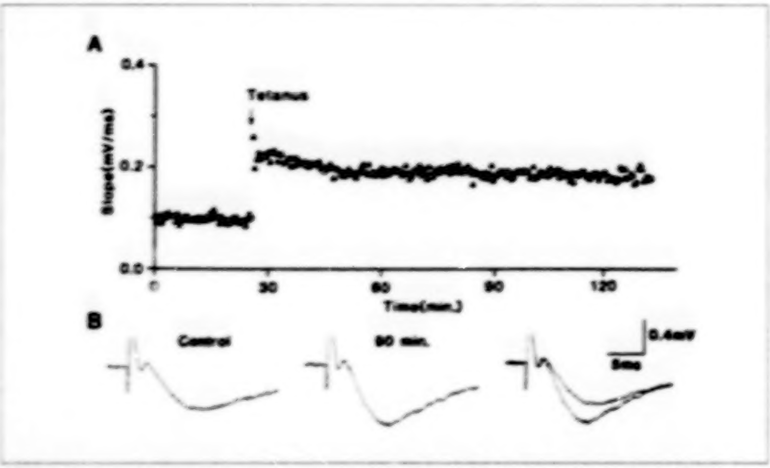

Figure 2. Graph showing long-term potentiation (LTP) in the CA1 region of the hippocampus. The slope of the excitatory postsynaptic potential (EPSP) recorded extracellularly in the dendritic region is plotted against time. The test stimulus was applied every 10 seconds. To elicit LTP, a 100 Hz stimulus for 1 second was delivered twice separated by 20 seconds. The stimulus intensity was doubled during the tetany. (From R. A. Nicoll, J. A. Kauer, and R. C. Malenka. 1988. The current excitement in long-term potentiation. *Neuron* 1:97–103. Copyright © 1994 by Cell Press. Used with permission.)

glutamate is released into the synaptic cleft. The glutamate then diffuses across the cleft and binds with proteins in the postsynaptic membrane, called receptors. Activation of these receptors depolarizes the postsynaptic cell. This transmission process, we think, is modified during learning. Figure 2 shows an example of the modification that occurs during the phenomenon referred to as long-term potentiation (LTP).

After obtaining a baseline with low-frequency stimulation, we can activate the synapses repetitively at high frequency for a brief period of time, a process not unlike what might be occurring in the brain when we were acquiring or learning new information. Immediately following the repetitive stimulation, the size of the response increases markedly and stays increased for the duration of the experiment. In intact animals, interestingly, this increased strength can last for weeks.

What occurs during the brief repetitive action that trips the switch and thus causes a change in the synaptic strength? And where does the change reside? A great deal of knowledge has been accumulated over the past decade about this process. Some of this knowledge is schematically diagrammed in figure 3. The glutamate released from the packets or vesicles in the presynaptic terminal

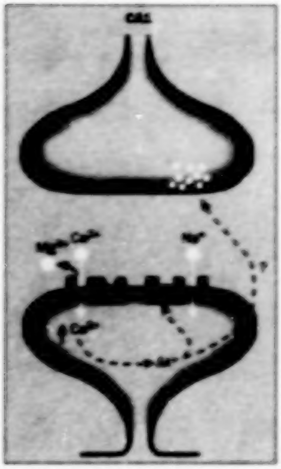

Figure 3. Summary diagram of long-term potentiation (LTP) in the hippocampus. The LTP found in the CA1 region is dependent on activation of NMDA receptors and a rise in postsynaptic Ca^{2+}. It is proposed that the rise in Ca^{2+} activates a process termed Δx, which probably involves CaM Kinase II. This enhances AMPA receptor function, but the generation of a diffusible signal that can feed back to the presynaptic terminal also may occur. (From R. A. Nicoll and R. C. Malenka. 1995. Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* 377:115–18. Reprinted with permission. Copyright © 1995 Macmillan Magazines Limited.)

bind to two types of receptors: AMPA and NMDA receptors. During the baseline transmission, the receptor responsible for depolarizing the cell is the AMPA receptor. It opens a channel that allows sodium to flow into the cell, causing the depolarization of the postsynaptic cell.

Although glutamate also binds to the NMDA receptor, this receptor does not contribute to the response during low-frequency stimulation. This is because Mg^{++} sits in the NMDA channel and prevents it from functioning. During repetitive activation, however, the cell depolarizes and magnesium is kicked out of the channel, allowing the channel to conduct. The NMDA channel, unlike the AMPA channel, has a high permeability to Ca^{++} in addition to sodium. The Ca^{++} serves as a trigger for the cascade of events that leads to the changes in synaptic strength.

There are two possible causes for this change. This calcium may initiate events that lead to the generation of another messenger that can go back and tell the presynaptic cell to release more transmitter. Or this synapse can become strengthened in response to a signal that can increase the number or enhance the ability of the AMPA receptors to generate responses.

The release of glutamate in the form of packets permits the investigator to record the all-or-none (quantal) response to a packet of glutamate. These packets, in addition to being released in response to a stimulus, also are released spontaneously at a low rate. It has been established at the neuromuscular junction that a change in the size of these quantal responses is due to a change in the postsynaptic sensitivity of the receptors. We have recorded these spontaneous events to address whether a rise in postsynaptic Ca^{++} increases the size of these events. To raise Ca^{++} at as many synapses as possible, we took advantage of the fact that the postsynaptic cell has voltage-dependent Ca^{++} channels in addition to NMDA channels. Repetitive activation of these voltage-dependent Ca^{++} channels causes a widespread rise in calcium in the postsynaptic cell.

Figure 4 shows the effects of Ca^{++} on the size of spontaneous quanta. A number of traces are superimposed and the downward deflections are the quanta. The top traces are from the control period and the bottom traces are shortly after strong activation of the voltage-dependent Ca^{++} channels. The quantal events clearly are much larger after the activation, and the time course is plotted in part B. This enhancement tends to decay over a 20–30 minute period.

It is unclear why this potentiation, unlike the LTP induced by NMDA receptor activation, is transient. Perhaps we are unable to raise Ca^{++} high enough by activating voltage-dependent Ca^{++} channels; perhaps some other factor is necessary for sustained potentiation. Nevertheless, these results indicate that a rise in postsynaptic Ca^{++} can increase the size of quanta, strongly suggesting that the postsynaptic sensitivity of AMPA receptors has increased.

How, then, does this calcium cause an increase in sensitivity in the postsynaptic cell? In addition to glutamate receptors, the postsynaptic density contains another set of proteins called kinases. Kinases attach a small molecule called phosphate onto other proteins; when they attach this phosphate, it changes the structure of these proteins and the function of the protein. One kinase, CaM Kinase II, is present in high amounts in the postsynaptic density. CaM Kinase II is activated by calcium and is ideally positioned to mediate the effects of Ca^{++} on AMPA receptor function. We have used specific blockers of CaM Kinase II and have found that the Ca^{++}-potentiation is markedly reduced by these inhibitors. We also have injected an activated form of CaM Kinase II into cells and this enhances AMPA receptor responses as does LTP.

In summary, we now know that the postsynaptic spine contains at least three distinct proteins: AMPA receptors, NMDA receptors, and CaM Kinase II. We postulate that long-term potentiation is

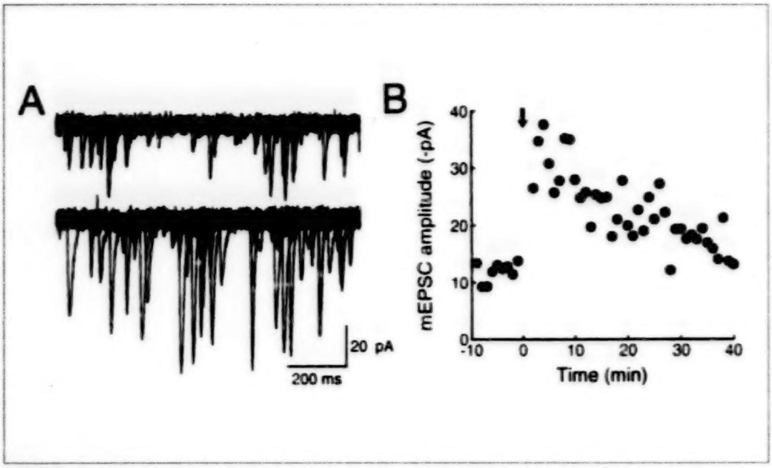

Figure 4. Voltage pulse-induced potentiation of miniature excitatory postsynaptic currents in a single CA1 pyramidal cell.

(A) In the upper trace, 10 current sweeps—each lasting 1 second—collected before the application of voltage pulses, are superimposed. In the lower trace, 10 current sweeps that were recorded around the peak of the potentiation are illustrated. It is clear that there is an increase in the amplitude of individual events following the voltage pulses. The cell was held at -80 mV.

(B) Plot of the same data illustrated in (A), but illustrating the mean mEPSC amplitude recorded during a 1-minute period. The mean control mEPSC amplitude is 12.6 pA, whereas after the voltage pulses the mean mEPSC amplitude increases to a maximum of 37.7 pA.

(From D. J. A. Wyllie, T. Manabe, and R. A. Nicoll. 1994. A rise in postsynaptic Ca^{2+} potentiates miniature excitatory postsynaptic currents and AMPA responses in hippocampal neurons. *Neuron* 12:127–38. Copyright © 1994 by Cell Press. Used with permission.)

a result, at least in part, of Ca^{++} entry through the NMDA receptor that results in the activation of CaM Kinase II, which in turn attaches phosphate onto the AMPA receptors. As a result, the AMPA receptor functions more effectively and generates a larger response in the postsynaptic cell.

Applications to Behavior

I wrote above that many investigators use a reductionist approach to study learning and memory. I have boiled down the problem to three proteins in the postsynaptic density: about as reductionist as one can get. There are tens of thousands of synapses on an individual neuron and millions of neurons in the hippocampus, so this clearly is a very reductionist approach. By taking the synapse apart and identifying some of the key players in the molecules involved in the synaptic changes, however, we should be able to obtain some insight we could take back to the behavior itself.

The NMDA receptor, for instance, is critically involved in generating LTP, the potentiation that we record electrophysiologically. If this electrophysiological event—this potentiation—has anything to do with memory, we would predict that by blocking this receptor, we should disrupt memory. In fact, there are compounds, called antagonists, that will block the NMDA receptors; indeed, one antagonist can impair learning in a rat very substantially. That such a linkage exists between physiology on one hand and behavior on the other is very reassuring.

From a clinical viewpoint, developing drugs that disrupt memory is not a particularly fruitful avenue to pursue. The drugs that block NMDA receptors, however, are of very great value in another condition in which nerve cells die during ischemia or lack of oxygen. When someone has a stroke, the ischemia releases an enormous amount of glutamate into the extracellular space. The excessive amount of glutamate acting on the NMDA receptor fills the cells with very high levels of calcium, and calcium at a high level kills cells. Using these antagonists for the NMDA receptor confers marked protection against many of the consequences—and much of the damage—that usually occurs during a stroke. Thus the NMDA receptor antagonists have value, but not for learning and memory.

Drugs targeted to the AMPA receptor might be a more fruitful approach. We have noted that some of the increase in synaptic strength occurs by enhancement of AMPA receptor function. What if we were able to design drugs that could mimic this enhancement of the AMPA receptor? This might be a strategy to improve the ability of an animal to learn or to restore function following loss of memory capacity. There is a class of drugs that specifically enhance the function of the AMPA receptor. Although they do it differently from the calcium-mediated process, the consequences are the same; that is, responses are enhanced. Gary Lynch and his colleagues have shown that when these compounds are injected into intact animals, they enhance the AMPA receptor function. More importantly, it appears that rats perform better in complex maze learning in the presence of these drugs. It is important to extend these results and determine whether this type of approach can be of value for Alzheimer's patients.

By investigating the brain through its smallest components, its molecular building blocks, we can gain important insight into the basic mechanisms that underlie very complex behaviors. Once we understand these building blocks, we can develop strategies and design drugs that can be applied to the complex behavior in a clinical setting. I am quite optimistic that this approach, enabling us to accumulate basic scientific knowledge rapidly, will give us the tools we need.

Suggested Readings

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Richard W. Tsien is the George D. Smith Professor at Stanford University. Born in China, Dr. Tsien is a naturalized U.S. citizen who received his bachelor's and master's degrees from the Massachusetts Institute of Technology. He was awarded a Rhodes scholarship in 1966 and spent four years at Oxford University, where he attained a doctorate in biophysics and served as a junior fellow in University and Balliol Colleges. Dr. Tsien then joined the faculty at the Yale University School of Medicine and began research on the fundamental mechanisms of the heartbeat and its control by ion channels and neurotransmitters. In 1988, he moved to Stanford to create a new Department of Molecular and Cellular Physiology.

Dr. Tsien researches the signaling mechanisms that link cellular electrical activity to cytoplasmic responses. To understand better complex signal processing in the brain, Dr. Tsien focuses on synaptic communication between neurons. Describing his work at the Library of Congress in 1995, he explained that synapses are fundamental elements of communication in the nervous system, and the ability of synapses to undergo marked changes in strength lies at the heart of the plasticity of the brain. "We can only expect," he said, "that fundamental knowledge about synaptic capabilities at cellular and molecular levels will prove important for deciphering how the brain functions as a whole. Understanding how the synapses work and the molecules behave may enable scientists both to apply that information to the entire organism and, ultimately, to find better ways of putting the knowledge to clinical use in treating human beings."

TAKING MEMORY TO BITS: NEUROTRANSMISSION AND PLASTICITY AT SINGLE SYNAPTIC NERVE ENDINGS

Richard W. Tsien

Several years ago, a theory about how memory takes place was featured in one of the leading scientific "journals" of our age, the *New York Times*. As shown in figure 1, the *Times* article included an illustration of the main components of the synapse, including the incoming neuron fiber, the presynaptic terminal, the presynaptic vesicles containing packets of neurotransmitter, the synaptic cleft, the postsynaptic receptors, and a postsynaptic spine filled with interesting signaling molecules.

The *Times* article summarized a then-popular theory proposing that memory traces are registered when the neck of the postsynaptic spine widens. According to this theory, a memory-initiating stimulus causes an entry of calcium ions, activation of a Ca^{2+}-dependent enzyme called calpain, and a change in the shape of the spine and its neck. As the neck widens, it was thought, electrical communication improved between the glutamate receptors on the spine head and the rest of the neuron. Synaptic strength thus would be increased in a specific and lasting way.

This theory proved extremely valuable as a stimulus for research. It drew attention to what might be called a Holy Grail of brain science: the goal of understanding the function of the brain at the level of individual synapses, in molecular terms. This is a logical and worthy objective, if an ambitious one. Synapses are fundamental elements of communication in the nervous system, and the ability of synapses to undergo marked changes in strength lies at the heart of the remarkable plasticity of the brain. If we can understand how the synapses work and the molecules behave, we may be able both to apply that information to the entire organism and, ultimately, to find better ways of putting the knowledge to clinical use in treating human beings.

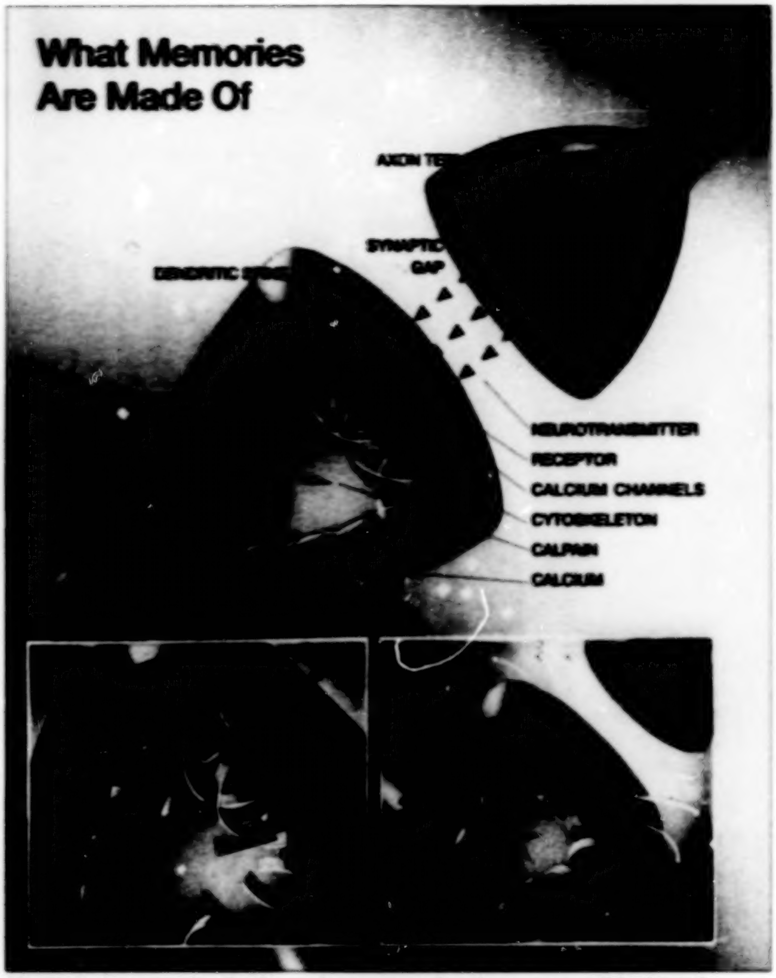

Figure 1. An example of a theory for memory that draws upon cell biological mechanisms, based on an original proposal by Dr. Gary Lynch. A cartoon representation of a synapse made up of a presynaptic axon terminal, the synaptic gap, and the dendritic spine of the postsynaptic (receiving) neuron. When surface presynaptic vesicles (purple spheres) release their content of neurotransmitter (blue arrows) into the cleft, the transmitter activates postsynaptic receptors (red pegs) that allow the entry of calcium ions (Ca^{2+}). The Ca^{2+} activates the Ca^{2+}-sensitive enzyme calpain, which disrupts the cytoskeleton and allows the spine to change its shape, converting the spine neck from a long, narrow shape into a short, wide passageway for electrical communication with the rest of the receiving neuron. (Art by Jim Ludtke. From George Johnson. 1987. Memory—Learning how it works. *New York Times Magazine*, August 9. Reprinted with permission.)

Recently, on a dreary late-evening flight back home to the San Francisco Bay area, I was trading stories with a fellow passenger, a Silicon Valley engineer, sharing my excitement about current theories about memory and how one might try to approach them experimentally. He seemed intent enough on understanding the neuroscience and even picked up some of the lingo. Sometime between the beverages and the meal service, he turned toward me, eyes wide, and declared: "I get it; you're working on the hardware problem!"

After some chuckling (I knew he was working on software systems), we compared observations about our respective colleagues, noting that in both fields we tend to divide ourselves up between "hardware" and "software": between those who prefer the "bottom-up" approach and those who like to work from the top down. Although analogies between computers and brains often are overstated, we agreed on the generalization that the consolidation of bottom-up and top-down approaches is always far more easily said than done.

In the case of neuroscientists, studies of synaptic "hardware" are sometimes pursued for their own sake, without much regard for their implications for what the "top-down" systems folks are doing. This is lamentable, but it is a fact of life. Nevertheless, the prevailing belief is that understanding basic mechanisms at the synapse level will both constrain and inspire "top-down" theories about memory.

Let us return to the spine neck idea of a few years ago. As it turns out, no one, including the original proponent of this hypothesis, believes it at present. The main problem with this hypothesis is that the spine neck is not narrow enough in its original state to impede significantly the electrical traffic between the head of the spine and the rest of the cell. Thus, widening the electrical highway, even if it did occur, would have little or no impact on the effective strength of the synapse. We have turned our attention to other kinds of explanations, of the sort mentioned by Roger Nicoll in his presentation, that call for modification of postsynaptic receptors or presynaptic transmitter release. But the spirit of the spine neck hypothesis is very much in line with the kind of cell biological answers that we now seek.

I would like to focus on some general issues about these mysterious synapses that are the center of so much attention. What are the functional capabilities of single presynaptic nerve endings? How many vesicles can be released by one terminal and how fast do vesicles recycle? Is the size of synaptic events constant or does it vary between individual nerve endings or between neurons? Such

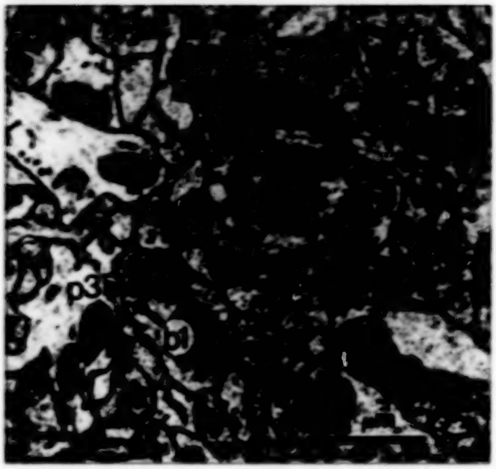

Figure 2. A cross section of brain tissue, as seen in the electron microscope. Numerous nerve terminals appear as sacks containing small circular vesicles (for example, those labeled with □ and △. Scale bar represents one-thousandth of a millimeter. (From K. E. Sorra and K. M. Harris. 1993. Occurrence and three-dimensional structure of multiple synapses between individual radiatum axons and their target pyramidial cells in hippocampal area CA1. *Journal of Neuroscience* 13:3736–48. Reprinted with permission.)

questions are fundamental to the flow of information between presynaptic and postsynaptic neurons. These now can be addressed, thanks to new methods that enable us to image individual synapses and study them one by one. New work in our Stanford University laboratory applies microstimulation techniques to individual presynaptic boutons (single synaptic terminals) to study their capabilities for releasing neurotransmitter and the ability of the postsynaptic receptor clusters to receive neurotransmitter. In addition, I will discuss a new way of studying synapses that have undergone an enduring change in strength, an approach somewhat akin to marking the synapses with an indelible ink.

The Virtues of Well-cultured Neurons

Until recently, much of our knowledge about synaptic transmission in the brain was based on extrapolation from classical experiments on the neuromuscular junction or more recent studies of mammalian brain slices. Unfortunately, there are major obstacles to the study of synapses in whole brain or even in thin slices of brain. A typical neuron receives tens of thousands or even hundreds of thousands of synapses from other neurons. Furthermore, individual synaptic nerve endings are very small—only about one micron

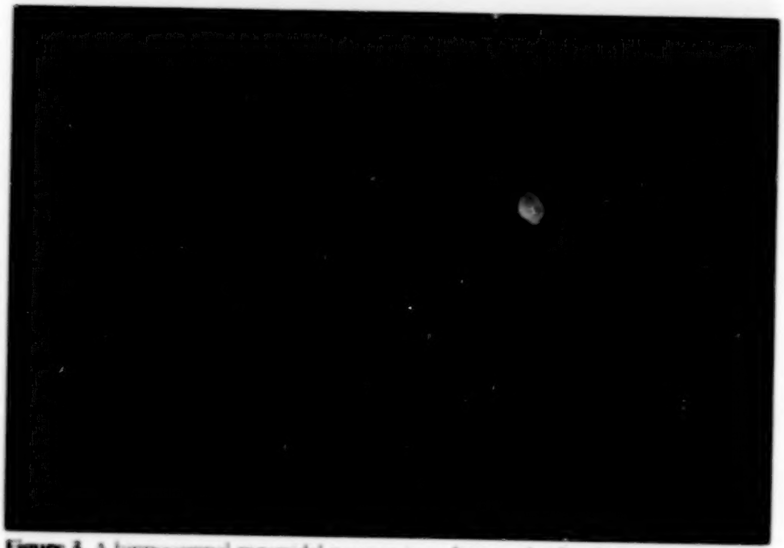

Figure 3. A hippocampal pyramidal neuron in a dispersed cell culture. Immunocytochemical staining of a presynaptic protein, synapsin, indicates the location of presynaptic terminals. (A. Malgaroli and R.W. Tsien, unpublished. Antibody to synapsin provided by Pietro DeCamilli, Yale University.)

across—and generally surrounded by other synapses, or other types of cells (glia) that appear to play a supporting role.

The thick, tangled nature of this neuroanatomical jungle becomes obvious in electron micrographs of brain tissue (figure 2). The electron micrograph is taken after the brain has been fixed, stained, and sectioned—after it is no longer living. In living brain tissue, individual synapses of this conventional morphology are currently inaccessible to direct physiological study. There is no way to visualize them and then stimulate them directly, one at a time.

One strategy that we and other neuroscientists have adopted to circumvent this problem is to take brain tissue apart, separating it into individual neurons, and then to allow the nerve cells to come together in tissue culture dishes to form synapses once again. Figure 5 shows a pyramidal neuron from the hippocampus, maintained in culture, that is synaptically connected. The synapses are labeled with an antibody to synapsin, an abundant presynaptic protein. If one looks at the ultrastructure of these synaptic connections, one sees the same kinds of presynaptic boutons, synaptic vesicles, and postsynaptic specializations as in the whole brain or the brain slice. Indeed, all indications are that physiological properties of synaptic transmission are very much the same in both systems, even though

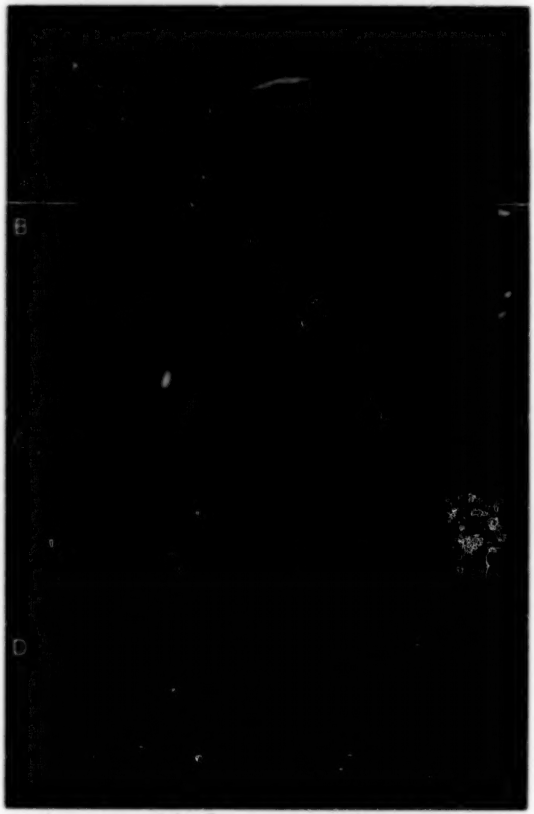

Figure 4. Chemical structure of the fluorescent membrane probe FM 1-43. (After W. J. Betz, F.-M. Mao, and G. S. Bewick. 1992. Activity-dependent fluorescent staining and destaining of living motor nerve terminals. *Journal of Neuroscience* 12:363–65.) Staining and destaining of synaptic terminals in cultured hippocampal neurons with the FM 1–43 dye.

(A) Light microscope image of a cultured hippocampal neuron extending between the edges of two glial cells (scanning DIC Nomarski optics).

(B) The same cell viewed in scanning fluorescence while being superfused in a KCl solution containing the styryl dye FM 1–43. Under these conditions, the probe labels the outer leaflets of essentially all of the cell membranes present. Note the presence of several small fibers extending over the cell body, which are not visible in the image.

(C) The same cell viewed in scanning fluorescence *after* an ~3-minute superfusion in normal saline following an ~60-second staining as in (B). Note the presence of numerous punctae of fluorescence that appear to be lined up along one of the neurites visible in (B). These fluorescent signals result from localized internalization of the dye and are not washed out in normal saline.

(D) The same field of view as in (C), after a 30-second superfusion in KCl-containing solution. Note the marked decrease in fluorescence intensity of most of the punctae visible in (C). Scale bar, 10 μm. (From T. A. Ryan, H. Reuter, B. Wendland, F. E. Schweizer, R. W. Tsien, and S. J. Smith. 1993. The kinetics of synaptic vesicle recycling measured at single presynaptic boutons. *Neuron* 11:713–24. Copyright © 1993 by Cell Press. Used with permission.)

the synaptic connections are less abundant. One of the advantages of working in culture is that the synapses are much easier to image and access for experimental manipulation. Thus, we can study synaptic communication in a more visual and immediate sense.

Visualizing Functional Synaptic Terminals

Working in culture, it becomes relatively easy to use a fluorescent dye to visualize functional boutons and the turnover of synaptic vesicles within them. Figure 4 illustrates the staining of the presynaptic terminals onto a cultured hippocampal neuron and their staining upon stimulation of vesicular release. This method was developed for nerve-muscle synapses by William Betz and his colleagues at the University of Colorado and extended to hippocampal synapses by Timothy Ryan and Harald Reuter in Stephen Smith's lab at Stanford. The fluorescent dye was named FM1–43 by its inventor, Fei Mao of Molecular Probes, a biotech company in Oregon.

The method works because synaptic vesicles in active synaptic boutons undergo a continual cycle of exocytosis and endocytosis (figure 5). Each vesicle can be thought of as a boxcar full of cargo in an ecologically minded railroad. Exocytosis, the dumping of the vesicular contents into the external medium, takes place by the formation of a pathway between the inside of the vesicle and the extracellular space. Endocytosis, the subsequent pinching off of the vesicle from the surface membrane, occurs in the aftermath of exocytosis and precedes the refilling of the vesicle with neurotransmitter cargo. During the several seconds between exocytosis and endocytosis, temporary continuity is established between the external medium and the interior of the vesicle. This connection provides a window of opportunity for movement of dye molecules into the vesicle, whereupon they immerse themselves up to their waists in the vesicle membrane and become highly fluorescent. After endocytosis, the dye molecules remain trapped in this state, marking the vesicle. So long as the vesicle remains intact in the nerve terminal, it will continue to be marked, even after the dye is removed from the bathing solution. If the vesicle undergoes another round of exocytosis, however, the trapped dye can escape and the marking of the vesicle will be reversed.

Capabilities of Single Nerve Terminals to Release Transmitter

Recent electrophysiological studies by Guosong Liu in our laboratory have shown close correlation between the loss of previ-

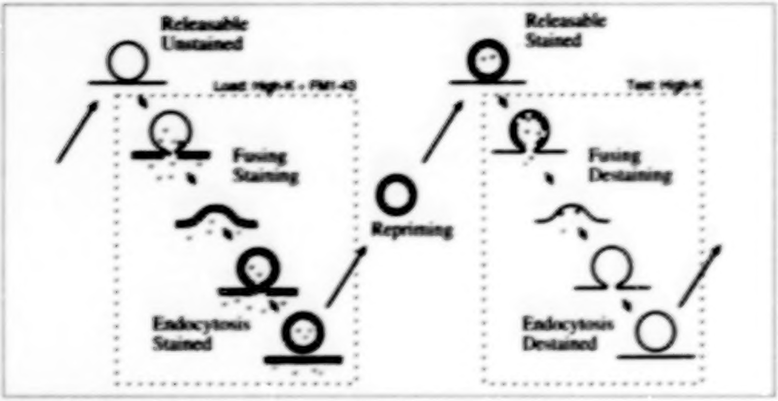

Figure 5. Individual steps in the recycling of synaptic vesicles.

(left) Vesicles may be labeled by virtue of the exposure of their luminal surfaces to the extracellular space upon fusion with the plasma membrane (exocytosis) during membrane depolarization. Once the vesicle has fused, dye in solution or on the outer leaflet of the plasma membrane labels the newly exposed vesicle membrane. The luminal surface of the vesicle is exposed to the extracellular space over a mean interval of 60 seconds. The nature and duration of the actual fused state is not known and is shown only schematically. After a mean access time to the extracellular space of ~60 seconds the vesicle is internalized (endocytosis), trapping within its volume the fluorescent membrane probe.

(right, top) After the extracellular dye is washed away, a pool of labeled vesicles remains as indicated by a fluorescent signal which is completely resistant to washing. These vesicles become available again, on a time scale of <~ 30 seconds, to undergo exocytosis upon an additional membrane depolarization (repriming). During such exocytosis, reestablishment of continuity of the vesicular lumen with the external medium allows the trapped dye to escape. (From T. A. Ryan.)

ously trapped dye—an optical index of vesicle exocytosis—and the occurrence of synaptic transmission: exocytosis physiologically detected by the postsynaptic cell. In Liu's experiments, boutons are focally stimulated by application of brief pressure to a micropipette to eject calcium-containing, potassium-rich solution (figure 6). This solution changes the electrical potential across the presynaptic membrane, which causes Ca^{2+} channels to open, allowing a local influx of Ca^{2+} ions that triggers neurotransmitter release. Evoked synaptic events were recorded as electrical currents transmitted to a recording microelectrode on the cell body.

As illustrated, when the puffer pipette was directed onto a bouton, there was a burst of synaptic events, but the number of evoked synaptic events was greatly reduced by moving the pipette by only a few thousandths of a millimeter to either side. This is one indication of the focal nature of the stimulation. Further reassurance is provided by FM1–43 flourescence, which shows loss of dye from

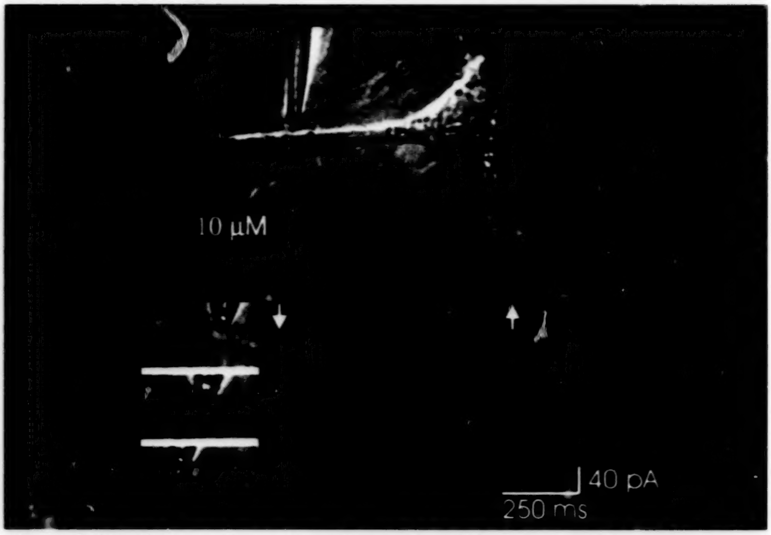

Figure 6. Stimulation and recording of synaptic activity from single boutons, identified as individual puncta of FM1–43 fluorescence.

(top) Fluorescence image of FM1–43 dye labeling of presynaptic boutons superimposed on a differential interference contrast image of a postsynaptic hippocampal neuron in cell culture.

(bottom) Evoked synaptic current response depends steeply on position of stimulating pipette. Vertical arrows indicate onset and termination of an application of a concentrated K+ solution. Applied solution contains 2 mM Ca^{2+}, while the rest of the cell is bathed in nominally 0 Ca^{2+} solution. Maximum response (middle trace) was obtained when stimulating pipette was centered on the presynaptic terminal structure. Small lateral movements greatly decreased the efficacy of the stimulus (upper and lower traces). (From G. Liu and R. W. Tsien. 1995. Properties of synaptic transmission at single hippocampal synaptic boutons. *Nature* 375: 404–8. Reprinted with permission. Copyright © 1995 Macmillan Magazines Limited.).

a bouton under direct stimulation, but not from another bouton a few thousandths of a millimeter away (figure 7). Thus, we are able to look at presynaptic release at the level of single visualized boutons, provided that the nerve terminals are not too dense.

When single boutons are stimulated frequently—say, once every two seconds—the number of synaptic responses per trial gradually declines (figure 8). We interpret this as a depletion of the available vesicles. If the bouton is given a rest period without stimulation—say, five minutes—the entire response to repetitive stimulation can be reproduced. If we vary the rest period, we find that there is a graded recovery of the ability to respond to stimulation. It takes about forty seconds on average for a vesicle that has just supported transmitter release to become available once again. During

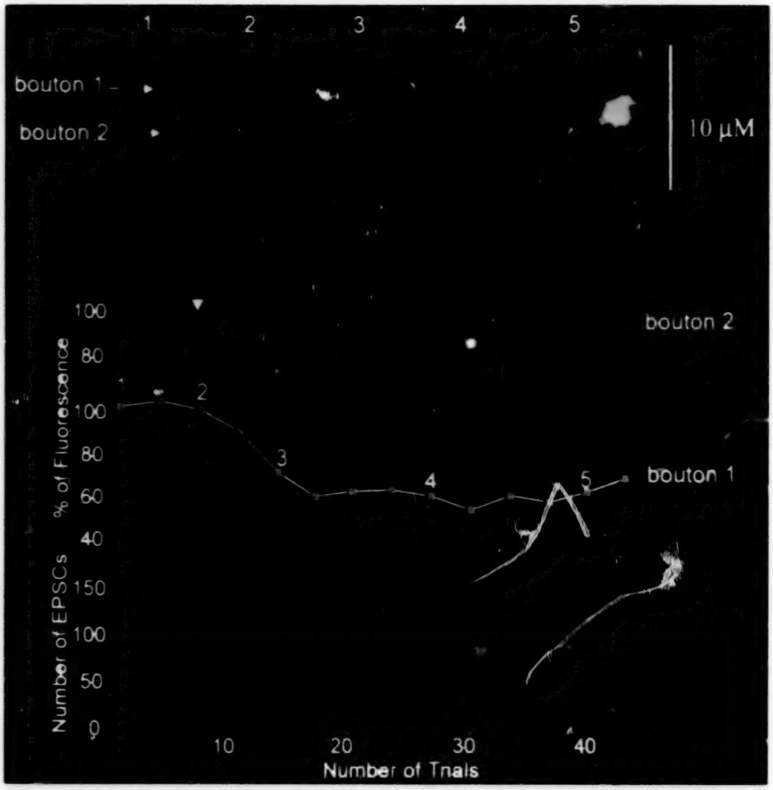

Figure 7. Localized stimulation of exocytosis. Puffer pipette was directed at the center of bouton 1, whose fluorescence fell markedly; whereas signal at bouton 2 remained essentially unchanged even after 50 trials of stimulation. Numbers correspond to specific time points as given below. The cumulative number of synaptic current events (bottom graph) parallels the destaining of bouton 1.

this time, the vesicle must pinch off from the surface membrane, regain its fill of neurotransmitter and any other necessary cargo, and rejoin the vesicles near the surface membrane once again. The number of synaptic events that can be detected over the course of depletion of the vesicular pool is about ninety on average. This is only a ballpark estimate, but it fits reasonably well with morphological counts of the number of vesicles in typical nerve terminals.

Variability of Individual Synaptic Responses

In the world of mass communication within the brain, the variability of the occurrence and strength of synaptic transmission is a serious issue. Variability is important because it sets limits on how reliably information can be transferred from one neuron to another.

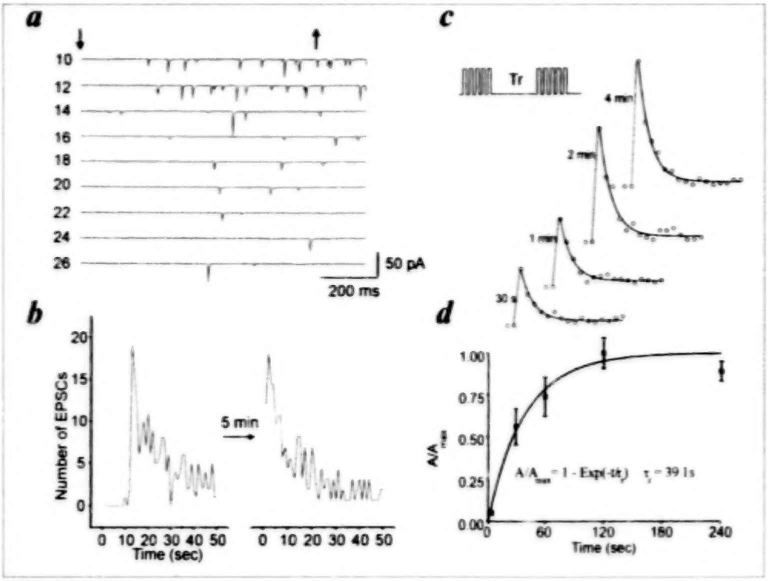

Figure 8.

(a) Declining response to repetitive stimuli. Puffer application repeated once every 2 seconds.

(b) Responses per trial plotted against time, showing that after such a decline, an ~5-minute rest period was sufficient to restore the response to repetitive stimulation to its original amplitude.

(c) Gradual recovery of responsiveness following complete depletion of vesicular pool in an individual bouton. Shown are averaged responses (n=3) following recovery intervals of 0.5, 1, 2, and 4 minutes.

(d) Pooled data for recovery kinetics in multiple runs from each of three individual boutons. The time course of recovery was determined by plotting the ratio of the response area after a variable rest period (A) to that after a 4-minute rest period (A_{max}). Single exponential fit (τ_r = 40 s). (From G. Liu and R. W. Tsien.)

Biological limitations on the reliability of synaptic transmission can take the form of transmission failures (the nerve impulse arrives, but no vesicle is released). Imagine running a laboratory or a congressional office where only half of the messages—letters, faxes, phone calls—were successfully received. Even when neurotransmitter release is successful, the responses to transmitter release from single vesicles appear to be highly variable in their electrical size.

We have examined the causes of this variability in response size by microstimulation of single nerve terminals. The variability persists even if we consider only those events arising from stimulation of the same individual synaptic bouton. A sizable percentage of responses are manyfold smaller than the largest responses. This

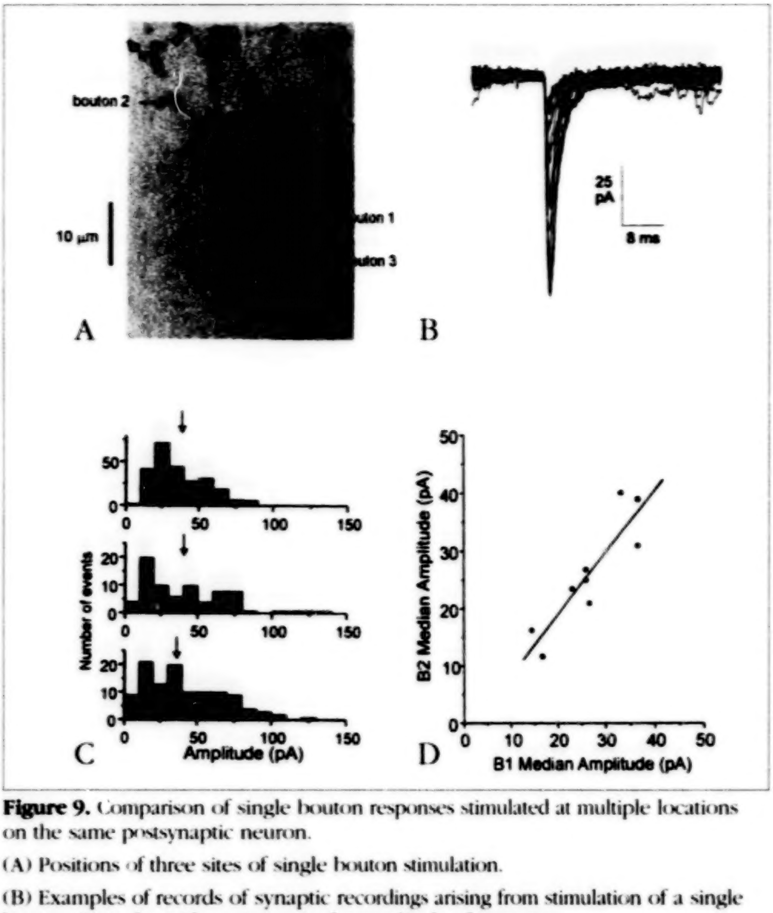

Figure 9. Comparison of single bouton responses stimulated at multiple locations on the same postsynaptic neuron.

(A) Positions of three sites of single bouton stimulation.

(B) Examples of records of synaptic recordings arising from stimulation of a single bouton. Note the wide variation in the amplitude of responses.

(C) Corresponding amplitude histograms of EPSCs evoked at the three sites. Note the similarity in the median amplitudes (vertical arrows) 36.5, 39.1, and 31.2 pA for boutons 1, 2, and 3 respectively.

(D) Data from pairs of boutons in five cells, and three boutons in two cells [e.g., (A), (B)]. Note the strong correlation between values of the median EPSC size at different sites on a given cell in the face of the large variation among different cells. (From G. Liu and R. W. Tsien.)

lack of consistency raises questions about how reliable information transfer can be at the level of single synapses. The good news came when we considered multiple synapses onto the same postsynaptic cell. Here we found that the average size of the response showed great consistency from one synapse to the next. If the average response was small at one synapse onto a cell, it was small for other synapses as well (figure 9). This indicates a kind of democracy in the sense that the average weight of input from individual synapses over time counts about the same. No segment of the postsynaptic neuron's constituency would be systematically neglected.

This homogeneity among synapses onto single neurons was remarkable, given that we found a fourfold variation in the average size of the synaptic events on different cells. The variation could be seen from one neuron to another in the same culture dish. Evidently, cells are able to control the average response size and have much latitude to do so. How was the wide variability across different neurons generated? We set out to find whether there was a difference in the behavior of postsynaptic receptors at individual synaptic sites. Minute puffs of solution containing the neurotransmitter (glutamate) were directly applied at synaptic locations labeled by the FM1–43 dye. Responses to the applied glutamate were always somewhat bigger than the synaptic signals at the same synapse, but the larger the glutamate response, the larger the synaptic signal. Thus, neuron-to-neuron variation was explained by the behavior of postsynaptic receptors at individual synaptic sites. Our most recent studies have focused on the mechanism of the adaptation. In all likelihood, the different response sizes are generated by different types of glutamate receptors, as indicated by differences in their physiological properties, particularly their rate of desensitization (the fading of responsiveness during a long-lasting application of neurotransmitter).

Significance of Plasticity in Postsynaptic Responses

What is the biological significance of the variation in postsynaptic responsiveness? Early in his experiments, Liu noticed a strong inverse correlation between the average response size and the density of synapses. A cell with sparse innervation by presynaptic terminals, indicated by staining with the FM1–43 dye, had a large average synaptic response, whereas a cell with a large density of synapses had a small synaptic response. We suggest that the postsynaptic neuron may be regulating the expression of glutamate receptors to compensate for the amount of synaptic stimulation it receives. Cells that receive a small number of inputs can afford to give each of them a strong weight, but as the density of synapses increases, they turn down the "volume control" on the individual inputs. This kind of compensation would keep the overall input within reasonable bounds, avoiding a state of perpetual excitation. Continued excitation is worth averting since it would nullify the

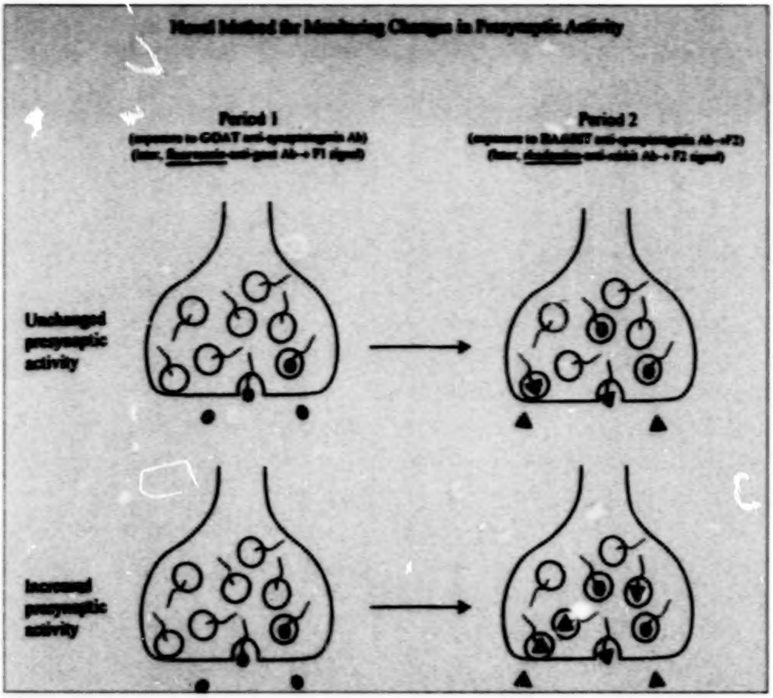
Novel Method for Monitoring Changes in Presynaptic Activity
Period 1
(exposure to GOAT anti-synaptotagmin Ab)
(later, fluorescein-anti-goat Ab→F1 signal)
Period 2
(exposure to RABBIT anti-synaptotagmin Ab→F2)
(later, rhodamine-anti-rabbit Ab→F2 signal)
Unchanged presynaptic activity
Increased presynaptic activity

Figure 10. Rationale behind use of antibody uptake to delineate changes in the degree of synaptic activity. Vesicles within a nerve terminal are allowed to take up antibody molecules directed against a region of the abundant synaptic vesicle protein synaptotagmin (syt) that protrudes into the vesicle interior.

(left) Uptake of a goat anti-syt antibody during period 1 yields a green color when later counterstained with a secondary anti-goat antibody that is fluorescein-labeled.

(right) Uptake of a rabbit antibody during period 2 yields a red color when later exposed to a rhodamine-labeled anti-rabbit antibody. If synaptic activity remains unchanged during period 2 relative to period 1, the uptake of red-stained rabbit antibody should match the uptake of green-stained goat antibody, all other factors being equal. A very different ratio of red to green would be expected if there was synaptic potentiation during period 2 relative to period 1. (Concepts developed in a study by A. Malgaroli, A. E. Ting, B. Wendland, A. Bergamaschi, A. Villa, R. W. Tsien, and R. H. Scheller. 1995. Presynaptic component of long-term potentiation visualized at individual hippocampal synapses. *Science* 268:1624–28.)

cell's usefulness as a decision-making device and almost certainly would result in massive entry of calcium and eventual cell death.

Beyond the implications for synaptic signal processing, insights about adaptive changes in the strength of individual synapses raise interesting questions about cellular mechanisms. Somehow the neuron must be informed about its density of innervation so that it may regulate the properties of its postsynaptic receptor clusters. This must involve changes in the pattern of expression of key genes. A challenge for the future is to understand signaling between synapses and gene expression at the nucleus. Such signaling must have broad impact on the fundamental behavior of neurons, including their resting membrane properties and spike-firing capabilities, as well as their synaptic responsiveness.

Visualizing Synaptic Plasticity of Individual Synaptic Boutons

One central question in the field of synaptic plasticity is how synapses change their activity over periods of hours or longer. Those of us in this field are continually challenged by how to take studies of synaptic plasticity to the level of individual synapses, and how to distinguish unambiguously presynaptic and postsynaptic changes in synaptic function. One approach, whereby single presynaptic terminals are visually marked according to their level of activity, has been developed in a very fruitful collaboration between Antonio Malgaroli and his colleagues at the University of Milan, and Anthony Ting, Richard Scheller, and myself in our department.

Our strategy depended upon the same transient connection between the vesicle interior and external medium that formed the basis of the fluorescent FM1–43 dye method (figure 10). To obtain a permanent record that vesicular exocytosis had occurred, we used a cell biological tag that indelibly labels the vesicle interior (a little like those permanent marker pens that always seem to leak in one's shirt pockets), rather than one that washes out. Changes in the level of synaptic activity were registered by use of two different stains: one stain for a baseline period (colored green in figure 10A), and another stain for a subsequent test period (colored red).

The tags that we actually use are two antibodies, both specific for the abundant synaptic vesicle protein synaptotagmin (syt), or more accurately, a specific region of synaptotagmin that protrudes into the vesicle interior. A goat anti-syt antibody yields a green color when later counterstained with a secondary anti-goat antibody that is fluorescein-labeled, while a rabbit antibody yields a red color when later counterstained with a rhodamine-labeled anti-rabbit antibody. The ratio of red to green stain is an index of the relative amount of synaptic vesicle cycling in the test and control periods. If, as it turns out, the two types of staining are about equally efficient, the ratio of red to green should be near one for nerve terminals whose activity remains constant (upper row), and greater than one for the terminals that undergo a significant increase in synaptic activity (lower row).

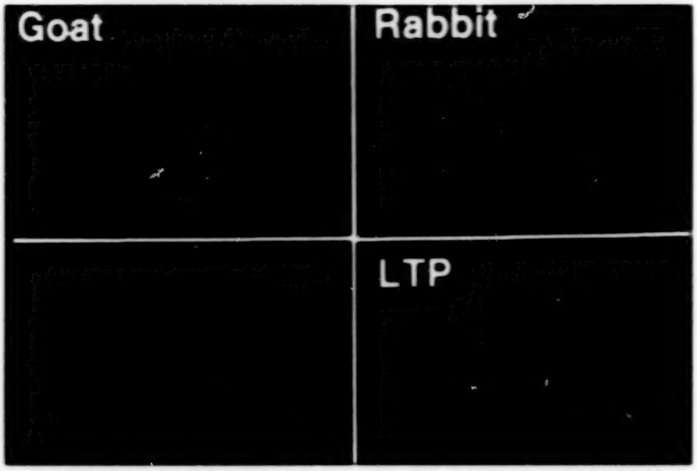

Figure 11. Visualization of the enhancement of vesicular turnover at individual boutons.
(left panels) Localized uptake of goat antibody, during 1-hour control periods, two different experiments.
(right panels) Uptake of rabbit antibody during subsequent 1-hour test periods.
(top right) After sham intervention.
(bottom right) After chemical stimulus to induce sustained potentiation ("LTP").

Control experiments demonstrated that the two kinds of marking work independently of each other. This makes sense because each vesicle contains many copies of synaptotagmin, so that the protein domains that interact with antibody are far from fully occupied by either antibody. Changes in presynaptic function caused by well-known maneuvers produced an appropriate change in the red/green ratio. Thus, we were able to show that long-lasting changes in the strength of synaptic transmission can be associated in an unambiguous way with an increase in presynaptic vesicular turnover. As a result of a form of synaptic long-term potentiation evoked by application of the neurotransmitter glutamate, the red/green ratio was increased by something between two- and threefold (figure 11). This confirms previous evidence that presynaptic function is enhanced during long-lasting forms of potentiation of synaptic transmission between hippocampal neurons.

The main advantage of this cell biological method is that the properties of the presynaptic terminals are assessed directly, without recourse to the responses of the postsynaptic cell, whose interpretation is often open to question. Another attraction of this approach is that potentiation can be studied one synaptic terminal at a time.

Conclusions

One very exciting aspect of the research endeavor in the Decade of the Brain is the area where neuroscience meets cell biology. The ability to study individual, visualizable synapses one at a time in neurons in culture opens up new possibilities for applying techniques of cell biology and cell physiology that are currently difficult—if not impossible—in the intact brain or even in thin slices of brain tissue. I have illustrated this with two sets of experiments that delineate basic properties of single excitatory synapses between hippocampal neurons and demonstrate changes in presynaptic vesicular turnover at individual terminals. It is to be hoped that "bottom-up" studies of the molecular and cellular properties of synapses will complement the "top-down" approaches of systems neuroscientists. We can only expect that fundamental knowledge about synaptic capabilities at cellular and molecular levels will prove important for deciphering how the brain functions as a whole.

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